

中華民國比較病理學會第二十二次比較病理學研討會
(新興人畜共通傳染病專題)

議程表

時間：中華民國九十年七月十五日（星期日）上午 08:30~下午 04:30

地點：行政院農委會家畜衛生試驗所 地址：台北縣淡水鎮中正路 376 號

主辦單位：中華民國比較病理學會 行政院農委會家畜衛生試驗所

中華民國獸醫病理學會 台灣法醫學會 展杏文教基金會

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時 間	議 程	主持人/演講人
08:30- 09:00		報 到
09:00- 09:20	致 詞	黃文哲 理事長 林士鈺 所長 吳福明 理事長 方中民 理事長 阮正雄 董事長
09:20- 10:00	立百病毒(Nipah virus)感染	美國疾病管制中心 謝文儒博士
10:00- 11:00		Coffee Break
11:00- 11:30	台灣動物鉤端螺旋體病 (Leptospirosis)血清流行病學調查	台灣大學獸醫學系 潘銘正教授
11:30~12:00	台灣人類鉤端螺旋體感染病例分析	長庚大學及林口長庚紀念醫院 楊智偉教授
12:00- 13:30	午餐 (中華民國比較病理學會理監事聯席會議)	
13:30-14:10	傳染病之病理學研究	美國疾病管制中心 謝文儒博士
14:10- 14:50	西尼羅河病毒 (West Nile virus)感染	美國疾病管制中心 謝文儒博士
14:50-15:20	休息	
15:20- 16:00	南美洲鉤端螺旋體病	美國疾病管制中心 謝文儒博士
16:00- 16:30	綜合討論	黃文哲 理事長 林士鈺 所長 吳福明 理事長 方中民 理事長 阮正雄 董事長

Nipah Virus Infection: Pathology and Pathogenesis of a New, Emerging Paramyxovirus Infection

Wun-Ju Shieh, MD, MPH, PhD

Abstract

In 1999, an outbreak of acute encephalitis among pig handlers in Malaysia led to the discovery of a novel paramyxovirus named Nipah virus. A multidisciplinary approach that included histopathology, immunohistochemistry, electron microscopy, virology and serology was pivotal in the investigation and diagnosis of this new infection. Clinical and autopsy findings were derived from a series of 32 fatal cases of Nipah virus infection. Routine histologic stains, immunohistochemistry and electron microscopy examined autopsy tissues. The main histopathologic findings included a systemic vasculitis with extensive foci of thrombosis and parenchymal necrosis particularly in the central nervous system. Endothelial cell damage, necrosis, as well as syncytial giant cell formation were seen in affected vessels. These pathologic changes were also found in most major organs examined except the liver. Neuronal viral inclusions observed under light microscopy have an ultrastructure composed of paramyxoviral nucleocapsid. Immunohistochemical analysis showed widespread presence of Nipah virus antigens in endothelial and parenchymal cells especially in the neurons. Widespread endothelial cell infection, vasculitis, thrombosis and neuronal infection in the central nervous system played an essential role in the fatal outcome of human Nipah infection, and appear to be critical to the unique pathogenesis of this new disease.

Introduction

An outbreak of a previously unrecognized paramyxovirus infection that caused an encephalitic syndrome mainly among pig handlers occurred in Malaysia and Singapore late 1998 and early 1999. In Malaysia, the outbreak appeared to have started in pig farms in Ipoh, in the state of Perak, and was spread by the movement of sick pigs to a second epicenter about 160 miles south in the state of Negri Sembilan. Later, the infection spread to workers in an abattoir in Singapore where sick pigs originating from Negri Sembilan were held and slaughtered. It was reported that a total of 265 patients were infected with 105 deaths, a mortality rate of nearly 40%. Most patients presented with a severe acute encephalitic syndrome but some patients also had significant pulmonary manifestations.

In several patients in whom virus isolation was attempted from cerebrospinal fluid (CSF), a syncytium-forming virus was consistently detected. Electron microscopy (EM) examination revealed an enveloped virus with tubular nucleocapsids, which when negatively stained showed a “herring-bone” structure characteristic of the family Paramyxoviridae. Infected

culture cells, which reacted strongly by immunofluorescence to anti-Hendra antibodies, and the detection of anti-Hendra IgM antibodies in the serum and/or CSF, suggested the possibility of Hendra or a Hendra-like virus infection. Reports from preliminary autopsy findings showed that while the central nervous system (CNS) appeared to be a major target, other organs might also be affected. In the CNS, the main pathology appeared to be endothelial damage and syncytial formation and direct neuronal infection. Viral genomic sequencing provided further evidence that this virus is related to but not identical with Hendra virus. This new virus was subsequently named Nipah virus after *Kampung Sungai Nipah* (Nipah River Village) where patients from whom the first viral isolates were obtained lived.

This presentation is based on the clinical and autopsy findings of thirty-two patients who had died of Nipah infection. It describes the pathologic findings including EM and tissue immunolocalization of viral antigens in this infection. A hypothesis for the pathogenesis of Nipah encephalitis is based on these findings. It provides evidence that this is indeed a new, unique infectious disease, which is quite unlike most other viral encephalitides.

A series of thirty-two fatal cases of Nipah infection, which comprised all the cases autopsied from late 1998 to mid 1999, were drawn from five hospitals in Malaysia. Since a total more than 100 patients with the infection died, these autopsies represented about 30% of all fatalities. Fifteen cases were from the Seremban Hospital, three from the Kuala Lumpur Hospital, nine from the Ipoh Hospital, four from the University of Malaya Medical Center, Kuala Lumpur, and one from the Kelang Hospital. Medical records from the various hospitals were systematically reviewed, and demographic, clinical and other data were extracted. Out of the thirty-two autopsies, twenty-nine were full autopsies and three were brain-only autopsies. Tissues were fixed in 10% buffered formalin from a few days to several weeks and extensively sampled for routine processing and paraffin embedding. Tissue blocks were chosen for IHC after reviewing the H&E slides. Serologic tests to detect antibodies to Nipah virus were performed using both IgM and IgG assays and inactivated Hendra virus antigens.

Results

The age range of the patients was 13 to 75 years with a mean and median of 43 and 44 years respectively. The male to female ratio was 29:3. The prodrome, defined as onset of fever to the day of hospital admission, averaged about 3.3 days, and ranged from one to 7 days. The duration of illness defined as onset of fever to death averaged 9.5 days, and ranged from two to 34 days. Four patients survived more than 14 days before death. One case that apparently had a similar clinical illness a few months before the present admission was presumed to have relapse encephalitis rather than acute encephalitis as in the other patients. Consequently, he was excluded from the estimation of the duration of prodrome and of illness.

Fever was found in all patients. More than 70% of patients complained of drowsiness, headache, and disorientation or confusion. The most frequent clinical sign elicited from patients was reduced consciousness. One case was recovering in the ward from encephalitis when he developed massive fatal intracerebral hemorrhage.

Histopathology

The pathologic features are unique in several aspects. The macroscopic appearance of the CNS and other organs was generally rather non-specific. In the CNS, lesions were generally difficult to identify macroscopically. However, in a few cases small discrete lesions may be found. The relapse encephalitis case showed a few small and discrete hemorrhagic areas measuring up to 5mm in the cerebral cortex. Out of ten brains examined for macroscopic evidence of increased intracranial pressure such as herniation, two had unequivocal herniation. One had tonsillar herniation and one had uncal herniation. Generally non-CNS organs were unremarkable but may show edema, congestion and focal hemorrhage.

Microscopically, pathological changes could be found in the blood vessels and parenchyma of multiple organs. There was extensive involvement of blood vessels in various organs including the CNS, lung, heart and kidney causing a multi-system disease in Nipah virus infection. Nonetheless, blood vessels in the CNS were the most severely involved. Typically, small arteries, arterioles, capillaries and venues showed evidence of severe vasculitis. Vasculitis was not found in medium-sized vessels such as the renal artery and vein, anterior and middle cerebral arteries, or large arteries such as the aorta and the pulmonary trunk.

Vasculitis was characterized by varying degrees of segmental endothelial destruction, mural necrosis and karyorrhexis. Mural necrosis often appeared fibrinoid. Sometimes there is only focal endothelitis. Inflammatory cell infiltration by neutrophils and mononuclear cells may be focal or circumferential, and partially or completely transmural. Thrombosis may be found in both vasculitic and uninflamed vessels. Necrosis and hemorrhage adjacent to vasculitic or thrombotic vessels were frequently seen.

Syncytial or multinucleated giant cell formation of the endothelium was found in about 27% of our cases, mostly in patients whose duration of illness ranged from 6 to 15 days. Typically, the syncytial formation consisted of several overlapping or sharply molded nuclei with moderate to abundant cytoplasm. The syncytium may protrude prominently into the vascular lumen. Syncytial cell formation was sometimes accompanied by vasculitis.

The major target system for Nipah virus infection is CNS. In the CNS, the main pathological findings were vasculitis and parenchymal changes including necrosis and viral inclusions. Extensive vasculitis and thrombosis involving gray and white matter could be found in the cerebrum, cerebellum, subcortical structures such as thalamus and caudate, brain

stem and spinal cord. There appeared to be no predilection for any particular region. The olfactory bulb, examined in nine cases, did not show any more pathological changes than were found in other areas of the CNS. Small meningeal vessels may also be affected.

In the CNS discrete, well-circumscribed and plaque-like necrotic lesions could be found in both the gray and white matter. These lesions, referred to as necrotic plaques, had diameters that ranged from about 200 microns to 5 mm or more. The smaller plaques were usually spherical or oval in shape. Vasculitic and/or thrombotic vessels, and a varying degree of edema could be found in the vicinity of the plaque in many instances. There were no large geographic infarctions of the type associated with occlusion of medium-sized or larger arteries such as the middle cerebral artery. In white matter plaques, damaged axons may form axonal spheroids similar to that seen in diffuse axonal injury. In the periphery of some plaques we found microcystic areas surrounding isolated, ischemia neuronal bodies. Isolated microcystic change may also be seen in areas where there were no adjacent plaques. In older necrotic plaques, there was a varying degree of inflammation in or surrounding the plaque. The inflammatory cells were usually mixed, and consisted of neutrophils, macrophages, microglial, plasma cells, lymphocytes and reactive astrocytes. Occasionally, micro abscesses may also be encountered. In some plaques, there may be a predominance of foamy macrophages. Elsewhere in the parenchyma there may be neuronophagia, microglial nodule formation and perivascular cuffing. Overall, parenchymal inflammation was present in 66% of cases.

Eosinophilic viral inclusions were found inside cytoplasm and nucleus of neurons, although nuclear inclusions were generally harder to find. Nuclear inclusions resembled the typical paramyxoviral inclusions, which occupied most of the nucleus except for a thin rim of chromatin at the periphery. Waxy cytoplasmic inclusions, which may be multiple in a single neuron, were usually small and discrete. Although inclusions were noticed in 63% of our cases, in many cases unequivocal inclusions were found in only a few neurons and only after a careful search. Most inclusions were found near vasculitic vessels or necrotic plaques.

Multiple foci of fibrinoid necrosis in the lung parenchyma each involving several adjacent alveoli were found in about 59% of our cases. These foci were often associated with small vessel vasculitis. Multinucleated giant cells, with or without nuclear inclusions, were occasionally noted in or lining the alveolar space adjacent to necrotic areas. In addition, alveolar hemorrhage, pulmonary edema, aspiration pneumonia were often encountered. Generally, bronchiolar epithelium was unremarkable except in one case in which a large bronchus showed severe transmural inflammation and ulceration. Vasculitis was noted in the heart in 31% of cases. A large myocardial infarction associated with vasculitis was found in a patient who had been comatose for more than 2 weeks. In another patient who survived more

than a month, focal myocardial fibrosis associated with vasculitis was noted. Renal damage occurred in 34% of cases and included vasculitis and focal glomerular fibrinoid necrosis with or without thrombosis and surrounding inflammation. The glomerulus may be totally destroyed, and replaced by a circumscribed inflammatory focus. More rarely, syncytial formation involving the periphery of the glomerulus and tubular epithelium may be seen.

The spleen typically showed white pulp depletion, and focal, acute necrotizing inflammation in the periarteriolar lymphoid sheath. In addition, one case showed large prominent multinucleated giant cells with intranuclear inclusions in the parenchyma. In the lymph nodes there may be reactive change characterized by large mononuclear cells, occasional necrosis and leucophagocytosis. Very occasionally, the rare multinucleated giant cell was encountered. Rare, focal vasculitis was also noted in the small mesenteric artery, adrenal gland, and pancreas. There were no apparent pathologic changes in the liver, thyroid, stomach and skeletal muscle in the cases examined.

Immunohistochemical Assay

IHC staining for viral antigen was positive in blood vessels in most of the major organs tested, particularly in tissues that showed vasculitis. No vascular staining was found in spleen or liver. Vascular staining was mainly seen in endothelium, endothelial syncytium and smooth muscle of the tunica media. No staining was present in medium-size or larger arteries. Vascular staining was no different in the gray or white matter of the CNS. In the CNS, apart from blood vessels, the most prominent staining was in neurons found in or around necrotic plaques, or near vasculitic blood vessels. Neuronal staining at the periphery of a necrotic plaque may form either a concentric or eccentric ring around it. Other patterns included focal staining at the plaque's edge, and a small, circumscribed zone of neuronal staining with no demonstrable adjacent plaque or vasculitis. Neuronal staining which may be granular or less commonly, homogenous, could be found in the nucleus, cytoplasm or peripheral processes of the perikaryon. Larger rounded or oval granules with smooth, well-defined edges probably represented viral inclusions as seen with H&E stains.

Necrotic plaques in the gray matter were more likely to show positive staining in the surrounding parenchyma compared to those in the white matter, since intra- or peri-plaque glial staining were rare. This was true even in cases in which neuronal staining was very strong. In the few cases where positive glial (astrocytes or oligodendrocyte) staining was obtained, it usually found in only a few cells. This relative sparing of glial cells was clearly seen in the putamen where "pencil bundles of Wilson" (white matter tracts), immediately adjacent to positively stained neurones, remained unstained. Similarly, in the pons there was general sparing of the white matter tracts in the anterior pons in contrast to positive pontine

nuclei staining. Occasionally, focal positive IHC staining of the ependymal and meningeal cells were found. The choroids plexus was negative for viral antigen in all cases.

Immunolocalization of viral antigen was clearly seen in the non-CNS organs although to a lesser extent compared to the CNS. For example, 24% of cases were positive by IHC in the lung and kidney compared to 84% in the CNS. In the lung, viral antigens were usually found in the areas of fibrinoid necrosis and in blood vessels. However, viral antigen was noted inside multinucleated giant cells in the alveolar space or in cells lining the alveolar space in 3 cases. Only one case had unequivocal staining of the bronchiolar epithelium, and was the same case with bronchial inflammation.

In the kidney, glomerular capillaries, small blood vessels and syncytial cells in the periphery of the glomerulus stained up for viral antigen. In the heart, staining was found mainly in the blood vessels, although in one case there was very focal myocardial staining adjacent to an area of capillary involvement. The occasional macrophage-like cell and multinucleated giant cell stained positive for viral antigen in the spleen and lymph node. The liver did not show any evidence of viral antigens. In the relapse encephalitis case, there was no IHC positivist in the non-CNS organs.

Serology

Elevated IgM antibody levels were found in more cases than IgG in both the serum and CSF. In the single largest group of 18 patients with a duration of illness of 6 to 10 days, IgM was found in the serum and CSF in 94% and 64% respectively, while IgG was found in 12% and 9% respectively. IgM antibodies generally appeared earlier than IgG, and earlier in the serum than CSF.

Correlation of IHC and Serology Assays as Diagnostic Tests

All the cases were examined by IHC, while serology was performed in serum and/or CSF in all but one case. The IHC analysis showed 87% of cases to be positive for viral antigen. 94% of cases were positive for serology in the serum and/or CSF. On the other hand, if both tests were performed together, confirmation of Nipah infection could be obtained in all the cases. There was an 81% correlation between positive IHC and serology results. Discrepancies between IHC and serology assays were found in 6 cases in which 4 cases were IHC negative and serology positive; 2 cases were IHC positive, out of which 1 case was serology negative and the other had no serology results unavailable.

Discussion

The characteristic histopathological features of Nipah infection were endothelial syncytial giant cell formation, vasculitis and viral inclusions particularly in the CNS. From the

diagnostic standpoint, perhaps the most unique histopathologic lesion in Nipah infection is the syncytial multinucleated giant cell in the endothelium. To our knowledge, hitherto, except for Hendra encephalitis, this feature has not been described in other infective encephalitides. Unfortunately, endothelial syncytium was not frequently encountered, occurring in only 27% of our cases. There is a greater chance for its detection if efforts are concentrated on small blood vessels, especially those in the CNS, and in cases that succumb early to the disease. Multinucleated giant cells of non-endothelial origin found in the kidney at the edge of the glomerulus appears to be a unique, albeit relatively rare, feature of Nipah infection. In contrast, multinucleated giant cells in the lung alveoli, splenic and lymph node parenchyma may also be seen in measles infection.

Systemic, disseminated vasculitis with predominant CNS involvement may be a useful feature for the diagnosis of Nipah infection. There are only a few other direct infective causes of vasculitis, including rickettsial, and more rarely, varicella-zoster and herpes simplex infections. In rickettsial encephalitis, vasculitis is usually subtler and vasculitis-associated thrombosis and microinfarction more focal. This is quite unlike Nipah encephalitis in which thrombosis and necrotic plaques (partly due to microinfarction) were often as widespread as vasculitis itself. Varicella-zoster and herpes simplex infections are associated with granulomatous anginal, features that are not seen in Nipah infection. The pattern of neuronal viral inclusions in Nipah infection, though by no means specific, highly suggests paramyxovirus infection. Other CNS changes such as perivascular cuffing, parenchymal inflammation and neuronophagia were rather non-specific features that may be found in other primary viral encephalitides.

Immunolocalization of viral antigen in various organs showed that CNS tissues were about 3-4 times more likely to be IHC positive than lung or kidney tissues, the next most likely organs to be positive. Thus, for diagnostic purposes, CNS tissues would be the most appropriate for IHC testing. Since there appears to be no predilection for specific sites, any part of the CNS should be more or less suitable. There was a good positive correlation of 81% between the serology and IHC as diagnostic tests for Nipah infection. In six cases in which there were discrepancies, all the four IHC negative cases had duration of illness of 14 days or more. This suggests that in some cases at least, most of the viral antigen may be cleared after 14 days or more following infection.

Overall, in cases where the duration of illness exceeds 14 days, serology appears to be slightly more sensitive than IHC, and therefore probably more useful than IHC particularly when the patients are still alive. The two IHC-positive cases in which serology was either negative or unavailable, underscore the important contribution of IHC to diagnosis. However, it is noteworthy that a combination of these two diagnostic tests enabled a positive diagnosis

in every case regardless of duration of illness. Other tests such as in-situ hybridization and polymerase chain reaction to detect the Nipah viral genome in tissues or suitable fluids from patients offer other possibilities for diagnosis and their contributions should be further investigated.

The available data point to endothelial infection and vasculitis as key events in the pathogenesis of acute Nipah infection. Direct infection, as evidenced by endothelial syncytium formation, localization of viral antigen and visualization of viral nucleocapsids in endothelium, presumably caused extensive endothelial injury and vasculitis, which in turn, resulted in disseminated thrombosis, vascular occlusion and microinfarction. This sequence of pathologic events was supported by the concomitant increasing frequency of syncytium formation, vasculitis, thrombosis, necrotic plaques and viral antigen in the CNS in the early phase of illness. Thrombosis was usually associated with vasculitis but may also be found in uninflamed vessels. The latter may be associated with focal vasculitis or possibly represented thromboembolism that had originated more proximally. Whatever its origin, thrombosis appeared to be severe enough in many vessels to cause complete occlusion leading to extensive necrotic plaques.

Apart from ischemia and infarction, direct viral infection may have contributed significantly to the cellular necrosis and degeneration in necrotic plaques as well since viral antigen and nucleocapsid could be localized in neurons found in and around plaques. Thus, both parenchymal ischemia/infarction and neuronal direct infection probably contributed significantly to the formation of necrotic plaques and the acute encephalitic syndrome. However, their relative importance and contribution is unclear.

Differing endothelial susceptibility to viral infection could account for the different frequencies of vasculitis in various organs: CNS, 80% of cases, followed by the lung, heart and kidney with 62%, 31% and 24% respectively. It is interesting to note that no vasculitis was apparently found in the spleen or liver. Our data showed that small vessels such as small arteries, arterioles, venules and capillaries were more prone to vasculitis and thrombosis than larger vessels. This is consistent with the general absence of large geographic infarctions, which would have implicated obstruction of larger blood vessels. Certainly, in the CNS there was a paucity of vasculitis in large vessels such as the middle cerebral artery. Large infarctions were also not found in non-CNS organs, with the possible exception of the single case of large myocardial infarction assumed to have resulted from vasculitis. Overall, in most cases, the frequency of vasculitis seemed to be proportional to necrosis and necrotic plaques.

Over and above the possible mechanism of vascular injury facilitating extravascular parenchymal infection, there seemed to be a viral predilection for certain cell types as well. In the CNS for example, neurons appeared to be particularly susceptible to infection, compared

to glial cells such as ependymal, oligodendrocytes and astrocytes. Interestingly, in the relapse encephalitis case, many more glial cells in the white matter, and ependymal stained for viral antigen compared to acute cases. The reason for this is unclear and remains to be elucidated. Overall, the virus seems to have a far lower predilection for epithelial cells compared with endothelial or other parenchymal cells notably neurons. Epithelial cell involvement included bronchiolar mucosa, renal tubule and probably podocytes at the edge of the glomerulus.

Although the above hypothesis is based on fatal cases, we believe that this pathogenetic mechanism also obtains in non-fatal cases. Clinically, non-fatal cases also suffer from a similar acute encephalitic syndrome, albeit less severely than fatal cases. Brain magnetic resonance imaging (MRI) studies of both non-fatal and fatal cases showed similar scattered, small and discrete lesions that were thought to represent necrotic plaques. More studies on animal models could further clarify the pathogenesis of Nipah infection.

The outbreak of Nipah virus infection in Southeast Asia was one of many emerging infections occurring worldwide recently including outbreaks of Ebola virus in Africa, Hendra virus in Australia and West Nile virus in North America. Initially in the Nipah virus outbreak, the clinical and laboratory speculation centered on Japanese encephalitis (JE) virus as the causative agent. However, several features of the outbreak argued against JE as the etiologic agent of this outbreak. Firstly, JE appeared unlikely in an outbreak that affected mainly adults rather than children. Secondly, most if not all patients had a history of direct contact with pigs. Thirdly, there was a clustering of cases and a higher attack rate in the same household than would be expected in JE. Finally, many patients had prior immunization to, and were therefore, likely to have developed protective immunity against JE.

It is now becoming clear that human infection most likely originated from pigs. Furthermore, the natural host for Nipah virus may be the fruit bat from the *Pteropus* family. Thus, in this epidemic, the pig may have acted as an intermediate or “amplifying” host rather than a natural host. The mode of virus transmission from pig to man is probably related to direct contact with these animals. Preliminary findings indicated that the rate transmission from patient to health care worker may be very low, an observation which correlated well with the relative rarity of lesions in the mucosal lining or alveoli of the respiratory tract, and renal glomeruli or tubules. Nonetheless, virus could be isolated from urine and tracheal secretions in some patients suggesting that human-to-human transmission is still possible. The possible mode of transmission of Nipah virus from bat to pig is still under investigation.

The emergence of novel paramyxoviruses such as Hendra and Nipah over a short period of a few years underscores the growing importance of this group of viruses as causative agents for previously unknown zoonoses. In the case of Hendra virus infection, first reported in 1995, the mode of transmission was thought to be from bat to horse to human, with the fruit bat as

the original natural host. Indeed, molecular characterization of Nipah virus has placed it closer to Hendra virus than any other member of the Paramyxoviridae family. This could partly explain some apparent clinicopathologic similarities between Nipah virus and Hendra virus infection. However, the pathology and pathogenesis of Nipah virus infection is probably unique. A greater understanding of pathology and pathogenesis of this new infection contributes significantly to the future development of therapeutic strategies and vaccines.

臺灣人類鉤端螺旋體感染病例分析

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楊智偉

Leptospirosis, a spirochetal infection, is considered the most widespread zoonosis, particularly in warm and humid climates. Infected animals shed urine in water or soil infecting human via abrasions wounds, mucosa, or swallowing of contaminated water. A broad spectrum of clinical manifestations may occur in humans when encountering pathogenic leptospira from animals. Risk factors of the infection include occupational exposure, recreational activities and households with close contact to animals. The clinical syndromes of leptospirosis vary from subclinical infection and self-limited anicteric febrile illness to severe and potentially fatal disease. Five to ten percent of leptospirosis infections induce multiple organ damage including kidney, liver and lung lesions. Weil's syndrome is a most severe form of the infection presented by febrile illness with hemorrhagic tendencies, hepatic dysfunction and acute renal failure, leading to fatality in a short time if not treated.

As a worldwide infection, endemic and epidemic spread of leptospirosis has caused morbidity and mortality and is considered a re-emerging infectious disease throughout the world, although the disease tends to be ignored in many cases. In Taiwan, leptospirosis has not been thought of as a common infectious disease for the past 20 years and appeared to be underestimated as a cause of acute renal failure until case report at Chang Gung Memorial Hospital in 1997. Since then an increasing number of leptospirosis cases have been diagnosed with the aid of the veterinary diagnostic facilities at the Graduate Institute of Veterinary Medicine, National Taiwan University. Tubulo-interstitial nephritis is the main cause of acute renal injury in leptospirosis. The pathogenic mechanism became clear when *in vitro* model was employed to dissect the role of the leptospira infection in tubulo-interstitial nephritis where leptospiral outer membrane protein may be responsible for the pathogenesis. This abstract gives a brief clinical summary of 12 patients with severe leptospirosis.

Patients with leptospirosis

Between May 1996 and August 1999, a series of 12 patients in Chang Gung Memorial Hospital with a mean age of 56.3 \pm 13.3 (28-77) years were diagnosed to have severe leptospirosis. Among these patients, six were farmers, one was an employee of a beef slaughterhouse, and two patients raised dogs as pets at home. There is a preference of the male gender (male 9, female 3).

Clinical presentations

Multiple organs were involved in these severe leptospirosis patients. The major presenting symptoms of leptospirosis were: fever (10/12), jaundice (10/12), and acute renal failure (12/12). Abdominal pain (8/12) and myalgia (7/12) were early symptoms. Splenomegaly occurred in 6 and hepatomegaly in 3 patients. Other associated presentations in these patients were: acute respiratory failure (9/12), consciousness disturbance (6/12), hemorrhagic tendency (4/12), rhabdomyolysis (3/12), and hemophagocytic syndrome (1/12), indicating a wide variety of leptospiral injury in multiple organs. Acute pancreatitis occurred in 3 patients presented with elevated serum amylase and lipase. Analysis of the biochemical laboratory data showed that the mean highest bilirubin level reached was 15.2 \pm 16.1 (0.4-42.2) mg/dl, the mean highest BUN level was 112.2 \pm 26.9 (74-144) mg/dl, and the mean highest creatinine level was 7.3 \pm 1.6 (4.8-9.5) mg/dl.

Diagnostic method and common pathogen in Taiwan - *Leptospira. shermani*

Diagnosis of leptospirosis depends on serologic methods via a microscopic agglutination test (MAT) to detect antibodies to leptospira, DNA detection by a polymerase chain reaction (PCR) and isolation of the microorganism. Serological diagnosis was made based on a single MAT titer more than 1:400 or a 2-week four-fold increase in titers against leptospira serovar tested. Among 270 serovars, *L. shermani* was the main infecting serovar (9/12) followed by one case each of *L. bratislava*, *L. balum*, and *L. copenhageni*.

Acute renal failure and thrombocytopenia

Acute renal failure was presented in all patients. Five patients had non-oliguria acute renal failure while 7 patients presented with oliguria acute renal failure. Hypokalemia was found on more than one occasion in 9 patients during admission. Among these patients, four required temporary intermittent hemodialysis, and one received continuous venous-venous hemodialysis (CVVHD) therapy. Acute tubulo-interstitial nephritis was diagnosed in two patients by renal biopsy. Although leukocytosis was found in 8 patients during the course of leptospirosis, 2 patients were leukopenic. As previously described by others, thrombocytopenia may be associated with severe endotoxin injury due to leptospirosis, and may appear in association with acute renal failure. In our series, thrombocytopenia occurred in 8 patients and, in particular, in all five patients who required hemodialysis. Three patients that required hemodialysis had severe thrombocytopenia (8,000, 12,000, 41,000).

Characteristic renal sonography findings

All patients received renal sonography studies at the acute renal failure stage. A

characteristic renal sonography finding in acute renal involvement of leptospirosis was swollen kidneys (mean 12.3+/-1.2 cm in left and 12.2+/-1.3 cm in right) and relatively normal parenchymal echogenicity. This finding may indicate tubulointerstitial edema by the invasion of the leptospira.

Renal tubular clearance test

To localize the renal tubular defect, tubular clearance tests were performed in four patients at the recovery phase of acute renal failure. The tests included: the bicarbonate infusion test, furosemide test and thiazide test, as previously described. Proximal tubular defects and incomplete renal tubular acidosis were found in one patient infected with *L. bratislava*. A medullary thick ascending limb dysfunction was found in two patients infected with *L. shermani* and normal tubular function was found in one patient recovering from infection by *L. shermani*.

Penicillin treatment rescue patients from mortality

Penicillin and tetracycline are the drugs of choice for leptospirosis treatment and may significantly improve multiple organ failure (Table 1). Several lines of evidence have shown that early treatment may rescue patients from multiple organ failure caused by leptospirosis. In our experience, treatment outcome for leptospirosis was very much favorable if initiated early by intravenous penicillin. Among 12 patients, eight survived because treatment was given early when leptospirosis was suspected. Penicillin treatment (mean 22.9+/-24.3 days) dramatically saved 7 patients from severe multiple organ failure. One of the surviving patients received tetracycline treatment because of the relatively mild leptospirosis. One penicillin-treated patient died because of irreversible severe multiple organ failure. Because of late recognition of the disease, three patients died without treatment. Besides the multiple nature of the organ involvement, the common presentations in deceased patients were: progressive jaundice, hepatic failure, severe renal failure followed by hypotensive shock, and coma. The Jarisch-Herximer reaction, a condition described for a temporary worsening condition after effective treatment due to lysis of organisms, occurred in one patient. However, supportive treatment rescued the patient.

Conclusion

Leptospirosis is a re-emerging infectious disease in Taiwan and worldwide. Increased alertness may help to identify patients from other causes of multiple organ involvement and rescue them early by antibiotics. Increased surveillance in the public health system may also help identify patients from areas where leptospirosis was not considered a frequent cause of

infectious disease.

Table 1. Clinical outcome of leptospirosis

Patient number:	12
Age:	56.3 (28-77)
Sex:	male 9; female 3
Animal exposure:	farmer 6; pet raiser 3; slaughterhouse worker 1
Multiple organ involvement:	12
Most common early presentation:	fever, jaundice, and acute renal failure
Outcome:	survived 8; expired 4
Penicillin treatment:	survived 7; expired 1
No penicillin treatment:	survived 1 (tetracycline); expired 3

INTRODUCTION OF ZOO NOTIC INFECTIONS (ZOO NOSIS)

Wun-Ju Shieh, MD, MPH, PhD

The World Health Organization defines Zoonosis as "Those diseases and infections, which are naturally transmitted between animals and man".

I. Impact of zoonotic infectious diseases

- 1) Severe and fatal Illness
- 2) Monetary loss and socioeconomic damage
- 3) Man-hours lost
- 4) Adverse effect on morale of personnel
- 5) Unfavorable publicity
- 6) Medicolegal implications

II. Epidemiological concepts

- 1) Incidental host - not required for the perpetuation of the organism.
- 2) Link host - bridges the gap between the maintenance host and man.
- 3) Amplifier host - increases the number of the infective agents (viruses and bacteria) to which man may be exposed.
- 4) A laboratory animal can be both a link host and an amplifier host.

III. Mode of transmission

- 1) Oral: contaminated food, water, milk, oral-fecal.
- 2) Direct contact – mucocutaneous.
- 3) Aerosol.
- 4) Vector bites.

IV. Factors influence the probability of disease transmission from animals to man:

- 1) Length of time the animal is infective.
- 2) Length of the incubation period in animals.
- 3) The stability of the agent. Most important in direct transmission, where the agent is exposed to environmental changes.
- 4) Population density of the animals in the colony.
- 5) Husbandry practices.
- 6) Maintenance procedures and control of wild rodents and insects.

- 7) Virulence and adaptation of the agent.
- 8) Route of transmission.
- 9) Genetic predisposition of host.
- 10) Immunity and host-parasite relationship.

V. Classification of zoonoses

A classification system based on the type of life cycle of the infective organism seems the most useful in planning a preventive medicine program. The World Health Organization Expert Committee on Zoonoses recommends the following categories:

- 1) Direct Zoonoses. Transmitted from infected vertebrate host to a susceptible vertebrate host by direct contact, fomented, or by a mechanical vector. No developmental change or propagation of the organism occurs during the transmission.

Examples: Rabies, trichinosis, and brucellosis.

- 2) Cyclozoonoses. Requires more than one vertebrate host, but no invertebrate host.

Examples: Human taeniasis and echinococcosis infections.

- 3) Metazoonoses. Agent multiplies, develops, or both in an invertebrate host before transmission to a vertebrate host is possible. (A definite incubation period must be completed before transmission.)

Examples: arboviruses, plague, and schistosomiasis.

- 4) Saprozoonoses. To transmit these infections a non-animal development site or reservoir is required, such as food plants, soil, or other organic material.

Examples: larva migrants and some of the mycotic diseases.

VI. Some examples of common zoonotic infections from selected animals

- 1) Diseases Acquired From Cats

Bacillus anthracis (Anthrax)

Bartonella henselae (Cat scratch disease)

Campylobacteriosis

Capnocytophaga canimorsus

Chlamydia psittaci (feline strain)

Leptospira spp. (Leptospirosis)

Pasteurella multocida

Rabies

Rickettsia felis

Salmonella spp. (Salmonellosis)
Toxoplasma gondii (Toxoplasmosis)
Visceral larva migrants
Yersinia pestis (Plague)
Yersinia pseudotuberculosis

2) Diseases Acquired From Cats

Bacillus anthracis (Anthrax)
Blastomycosis
Brucella canis
Campylobacteriosis
Capnocytophaga canimorsus
Cryptosporidiosis
Cutaneous larva migrants
Echinococcosis
Francisella tularensis
Granulocytic ehrlichiosis
Leptospira spp. (Leptospirosis)
Pasteurella multocida
Yersinia pestis (Plague)
Rabies
Rocky Mountain Spotted Fever
Visceral larva migrants
Yersinia enterocolitica

3) Diseases Acquired From Horses

Actinobacillus spp.
Bacillus anthracis (Anthrax)
Brucella app. (Brucellosis)
Cryptosporidiosis
Hendra virus
Leptospira spp. (Leptospirosis)
Rabies
Rhodococcus equi
Salmonella spp. (Salmonellosis)
Yersinia enterocolitica

4) Diseases Acquired From Cattle

Actinomyces pyogenes
Bacillus anthracis (Anthrax)
Brucella abortus
Cowpox
Coxiella burnettii (Q-fever)
Cryptosporidiosis
Escherichia coli O157:H7
European tick-borne encephalitis
Foot and mouth disease
Leptospira spp. (Leptospirosis)
Mycobacterium bovis
Prion disease (Mad cow disease)
Rabies
Salmonella spp. (Salmonellosis)
Taenia saginata
Yersinia enterocolitica

5) Diseases Acquired From Sheep

Actinobacillus spp.
Bacillus anthracis (Anthrax)
Brucella spp. (Brucellosis)
Chlamydia trachomatis (ovine)

Cryptosporidiosis

European tick-borne encephalitis
Francisella tularensis (Tularemia)
Giardiasis
Leptospira spp. (Leptospirosis)
Poxvirus (Orf)
Salmonella spp. (Salmonellosis)
Yersinia enterocolitica

6) Diseases Acquired From Pigs

Bacillus anthracis (Anthrax)

Brucella suis
Clostridium botulinum (Botulism)
Clostridium perfringens (Pigbel)
Influenza virus
Leptospira spp. (Leptospirosis)
Nipah virus
Pasteurella multocida
Rabies
Salmonella cholerae-suis
Streptococcus suis type 2 (group R)
Taenia solium (Cysticercosis)
Trichinella spiralis (Trichinellosis)
Yersinia enterocolitica

7) Diseases Acquired From Rodents

Borellia recurrentis (Tick-borne relapsing fever)
Francisella tularensis (Tularemia)
Guanarito virus (Venezuelan hemorrhagic fever)
Hantaviruses (Hantavirus pulmonary syndrome, Hemorrhagic fever with renal syndrome)
Junin virus (Argentine hemorrhagic fever)
Lassa fever virus (Lassa fever)
Leptospira spp. (Leptospirosis)
Listeria monocytogenes (Listeriosis)
Lymphocytic choriomeningitis virus
Machupo virus (Bolivian hemorrhagic fever)
Rabies
Rickettsialpox
Rickettsia typhi (Endemic typhus)
Salmonella spp. (Salmonellosis)
Spirillum minus (Rat bite fever)
Streptobacillus moniliformis (Rat bite fever)
Yersinia enterocolitica
Yersinia pestis (Plague)

8) Diseases Acquired From Rabbits

Brucella suis biotype 2

Coxiella burnettii (Q-fever)
Francisella tularensis (Tularemia)
Yersinia pestis (Plague)

9) Diseases Acquired From Rabbits

Campylobacteriosis
Ebola virus?
Entamoeba histolytica
Francisella tularensis (Tularemia)
Giardia lamblia (Giardiasis)
Hepatitis A
Herpesvirus simiae (B virus)
Marburg virus ?
Measles virus
Monkeypox virus (Monkeypox)
Mycobacterium tuberculosis
Salmonella spp. (Salmonellosis)
Shigella spp. (Shigellosis)
Simian immunodeficiency virus

VII. Important message

Physicians and veterinarians hold significantly different views about the risks posed by certain infectious agents and species of animals and communicate very little about zoonotic issues. Veterinarians should be involved in many aspects of zoonotic disease prevention, including patient education.

Communication between physicians and veterinarians about zoonotic diseases is largely absent. Enhancing such communication could help prevent transmission of zoonotic agents. Links between the professions on a broader scale (e.g., through combined veterinary/medical student training and continuing education) to foster a broader consensus about zoonotic disease risks and prevention should also be encouraged.

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VIRAL HEMORRHAGIC FEVERS

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INTRODUCTION

The combination of fever and hemorrhage can be caused by a diverse group of human pathogens, including viruses, rickettsiae, bacteria, protozoa, and fungi. However, the term hemorrhagic fever (HF) is usually reserved for systemic infections characterized by fever and hemorrhage caused by a special group of viruses transmitted to humans by arthropods and rodents. The syndrome was well-described in the early part of this century by scientists in the Soviet Union dealing first with hemorrhagic fever with renal syndrome (HFRS) and later reinforced by their experience with outbreaks of Crimean Congo HF (CCHF), Central Asian HF (later shown to be CCHF), and Omsk HF. These viruses persist in nature through zoonotic cycles, although in the case of dengue and sometimes yellow fever, human infection caused by the bite of a mosquito intermediate may be an important factor in disease maintenance. With the exception of the dengue viruses, all these agents have a degree of aerosol infectivity, necessitating special handling procedures usually in biosafety level 4 facilities. Because these viruses are extremely virulent and associated with disease outbreaks with high mortality rates, they have gained considerable public notoriety and are considered among the most threatening examples of what are commonly called emerging pathogens.

DEFINITION

Viral hemorrhagic fevers (VHFs) are febrile illnesses characterized by abnormal vascular regulation and vascular damage and are caused by small, lipid-enveloped RNA viruses. This syndrome, as well as pneumonia or other infectious disease syndromes, can be caused by a variety of viruses belonging to four different families (Table 1).

GEOGRAPHY AND EPIDEMIOLOGY

As a group, all recognized VHFs are zoonotic and cause a somewhat similar clinical syndrome. Infection is usually contracted from an arthropod or rodent vector; thus, the highest incidence and risk for disease occurs among individuals traveling to or residing in rural settings. Notable exceptions include the occasional occurrence of VHF in urban areas in association with reservoirs of Seoul virus (*Rattus norvegicus*), dengue viruses (*Aedes aegypti*), and urban yellow fever (*Aedes aegypti*). Several of these diseases can be spread from human to human and have been associated with nosocomial outbreaks among health care workers.

Hemorrhagic fever viruses are distributed worldwide, and the diseases they cause are

traditionally named according to the location where they were first described. The different viruses and associated HFs are more or less confined to distinct geographic areas (Table 1). However, changes in geographic patterns and the introduction of disease into non-endemic areas can occur because of multiple factors, including environmental conditions and air travel. For example, the importation of Rift Valley fever virus into Egypt led to major epidemics of Rift Valley fever in that country in 1977 and 1993.

MORPHOLOGIC FEATURES

The four families of viruses that cause HF syndrome differ in their genomic structure, replication strategy, and morphologic features. Arenaviruses, bunyaviruses, and filoviruses are negative-stranded, whereas flaviviruses are positive-stranded RNA viruses. All viruses have a lipid envelope that is acquired by budding at either the cell surface or the internal membranes. The size and shape of these viruses vary from relatively small (35-50 nm), uniform round particles, as seen with flaviviruses, to more pleomorphic, rod-shaped particles (measuring occasionally up to 15,000 nm) in the case of filoviruses.

CLINICAL FEATURES

VHFs typically begin with a febrile prodrome with myalgia occasionally accompanied by gastrointestinal disturbances. By the time medical attention is sought, patients usually have a severe, acute illness, with evidence of abnormal vascular regulation and vascular damage. Systemically, the abnormal vascular regulation manifests by mild hypotension in the early stages of the disease and by shock in more severe and advanced cases. Local vascular abnormalities are usually visible as conjunctival suffusion, flushing over the face and thorax, and various exanthemas. Vascular damage may be evident as capillary leakage in non-dependent areas, such as periorbital edema, a propensity to develop pulmonary edema after fluid infusions, proteinuria, or by the presence of effusions in serous cavities.

Thrombocytopenia is characteristic in most of the VHFs and is likely to be accompanied by hemorrhage, which may signal underlying vascular damage. The hemorrhage is usually petechial in nature and commonly seen on the skin and mucous membranes. In some diseases, there may be a period of remission of fever before recovery or progression to a more severe stage. Profuse bleeding, central nervous system disturbances, and frank shock usually accompany severe disease. Jaundice is a feature of yellow fever, but may also occur in severe Crimean Congo HF, Rift Valley fever, and filovirus HF. Renal compromise is usually proportional to the degree of shock, but in HFRS it dominates and is caused by primary renal lesions.

Hantavirus pulmonary syndrome (HPS) differs somewhat from the other diseases listed.

The prodrome is terminated by the onset of pulmonary edema. Hypotension and shock may be problematic even if hypoxia is corrected. Although thrombocytopenia is always present and abnormal PTT is common, petechiae or bleeding occur only in a small percentage of cases. Vascular and other major clinical manifestations are also largely confined to the thoracic cavity.

PATHOLOGY AND PATHOGENESIS

Although VHF share many common pathologic features, the overall changes vary among the different diseases. The similar pathologic and immunopathologic findings in cases of VHF suggest that microvascular involvement and instability is an important common pathogenetic pathway leading to shock and bleeding in many instances. Infection of macrophages and other cells of the mononuclear phagocytic system are also thought to play a critical role in the pathogenesis of VHF through the secretion of physiologically active substances, including cytokines and other inflammatory mediators. However, the details of the pathogenesis of VHF are poorly understood, and the differences observed among these diseases and the lack of significant anatomic lesions in some fatal cases emphasizes the need for more comprehensive studies.

Common pathologic findings at autopsy include widespread petechial hemorrhages and ecchymoses involving skin, mucous membranes, and internal organs. However, in many HF patients manifestations of bleeding may be minimal or absent. Effusions, occasionally hemorrhagic, are also frequently seen. Widespread, focal, and sometimes massive necrosis can be commonly observed in all organ systems and is often ischemic in nature. Necrosis is usually most prominent in the liver and lymphoid tissues. In addition, the kidneys frequently show evidence of acute tubular necrosis secondary to shock. In some instances the necrosis is a consequence of direct viral cytopathic effect. Although lymphoid necrosis and depletion are the general rule, proliferative changes of lymphoid tissues may be found in some diseases, such as in hantavirus-related illnesses. Erythrophagocytosis is also commonly seen in the spleen, lymph nodes, and liver of HF patients. Microvascular thrombosis can be seen in tissues of a small proportion of patients, and it is highly possible that disseminated intravascular coagulation (DIC) is important in the pathogenesis of some of the different HFs; however, definitive hematologic studies and pathologic evidence at autopsy are often lacking. Commonly observed histopathologic changes in the lung include various degrees of hemorrhage, interstitial pneumonitis, and diffuse alveolar damage.

There are relatively few, but excellent, original studies describing the pathologic features of certain HFs. With some diseases, not much has been reported mainly because of the risk of hemorrhage associated with biopsies and because of biosafety concerns during autopsy.

Several references containing detailed pathologic descriptions in human cases and experimental animal models are recommended (Table 3). The histopathologic features of the different VHFs are not pathognomonic, and immunohistochemistry and other laboratory tests are essential to confirm the diagnosis. In the remaining part of this section, the main histopathologic and immunopathologic features of some of these diseases will be briefly reviewed.

The histopathologic features in arenaviral infections, such as Lassa fever, Argentine HF (AHF), Bolivian HF (BHF), and Venezuelan HF, are strikingly similar. The most consistent microscopic feature is found in the liver and consists of multifocal hepatocellular necrosis with cytoplasmic eosinophilia, Councilman body formation, nuclear pyknosis, and cytolysis. Inflammatory cell infiltrates and necrotic areas are usually mild and, when present, consist of neutrophils and mononuclear cells. Immunohistochemical studies demonstrate hepatocellular infection in association with focal areas of necrosis. Extensive infection of macrophages and mesothelial cells lining several serosal surfaces is characteristic of arenavirus infections and helps explain serous effusions commonly seen in patients with these infections. Other pathologic features that may be seen in arenavirus infections include a mild interstitial pneumonitis, myocarditis, as well as reticuloendothelial tissue infection and damage. In CCHF and Rift Valley fever (RVF), the main histopathologic lesions are seen in the liver, spleen, and lung. The changes in the liver are similar to those seen in a number of VHFs and consist of widespread hepatocellular necrosis, usually midzonal in case of RVF, associated with variable degrees of hemorrhage and Councilman body formation. As in the case of Lassa HF, the inflammatory cellular response in necrotic areas is minimal or absent, and only a mild periportal mononuclear infiltrate is sometimes seen. Immunohistochemistry reveals only focal infection of hepatocytes, Kupffer cells, and sinusoidal lining cells. The predominant features in lymphoid tissue include sinusoidal dilatation and generalized lymphoid depletion. Lungs may show an interstitial pneumonitis and are usually congested with widespread intraalveolar edema and hemorrhage. Rift Valley fever, in addition to typical features, may cause encephalitis and retinal lesions in some patients.

Among the VHFs the filoviruses cause the most widespread destructive tissue lesions. The pathologic changes are similar in Marburg virus and Ebola virus infections, although the latter tends to be more severe. Necrosis is seen in many organs being maximal in liver, spleen, kidney, and gonads. The necrosis is both ischemic in nature and related to cytopathic effect of the virus. The most characteristic histologic features are seen in the liver with widespread hepatocellular necrosis, Councilman bodies, microvesicular fatty change, and Kupffer cell hyperplasia. The portal tracts usually exhibit extensive karyorrhectic debris and a mononuclear inflammatory infiltrate. Characteristic intracytoplasmic viral inclusions are seen

within hepatocytes. They are usually numerous, eosinophilic, and oval or filamentous in shape, and ultrastructurally are seen to be comprised of aggregates of viral nucleocapsids. The viral inclusions and distribution of antigens can be confirmed and studied by immunohistochemistry. Lymphoid tissues show extensive follicular necrosis and necrotic debris. Myocardial edema is seen but is not associated with any appreciable inflammatory infiltrates. The lungs are usually hemorrhagic and show features of diffuse alveolar damage.

The published descriptions of the pathology of Kyasanur Forest Disease and Omsk HF are few. Some of the features of individual diseases are summarized in Table 2. Generally speaking, microscopic appearances of fatal yellow fever and dengue virus infections are somewhat similar. The most consistent features are seen in the liver and consist of hepatocellular necrosis, Councilman bodies, and microvesicular fatty change. Although the acidophilic or Councilman body is of considerable diagnostic value in yellow fever, it is not pathognomonic of the disease and can be seen in the different HF as well as other hepatic diseases. In yellow fever the hepatic necrosis is extensive, midzonal in distribution, while in fatal DHF/DSS it is less severe and tends to be centrilobular or midzonal. In severe cases of both diseases the necrosis may extend beyond the midzone resulting in almost complete necrosis of the lobule; however, a rim of intact hepatocytes usually remains around the portal tracts and central veins. Follicular hyperplasia of spleen and lymph nodes can be seen in fatal dengue. Otherwise, with both diseases the histopathologic features in other organs are variable and resemble those of other VHF. Immunohistochemistry is extremely valuable in providing a definitive diagnosis and differentiating these infections from other VHF and diseases with similar histopathologic features such as viral hepatitis and leptospirosis.

DIAGNOSIS

The diagnosis should be suspected in any patient returning from an endemic area, particularly if there is travel to rural areas during seasonal or epidemic disease activity. There may be a history of exposure to the vector, tick exposure being particularly significant. Clinical features such as high fever, prostration, flushing, conjunctival injection, postural hypotension, axillary petechiae may be present early.

The clinical laboratory findings may be helpful (Table 4), although care should be taken with potentially infectious blood and other body fluids (VHF guidelines). Proteinuria is common to constant, depending on the disease. In hantavirus diseases the white count is elevated or at least shows a left shift; atypical lymphocytes are usually present with thrombocytopenia. Thrombocytopenia is also characteristic of all the HF with the exception of most Lassa fever patients who nevertheless have dysfunctional platelets. In the South American HF there is always a leucopenia.

The major diseases that must be clinically ruled out are malaria, rickettsial diseases, leptospirosis, shigellosis, and typhoid because all may mimic the HF and all are treatable. Empiric treatment may be indicated for one or more of these conditions. The usual differential diagnosis should be carried out considering other diseases to include the less common regional diseases (e.g., trypanosomiasis may be associated with thrombocytopenia) and the medical conditions, which are seen worldwide such as measles, lupus, hemolytic uremic syndrome, etc.

The diagnosis of VHF suspected by history and clinical manifestations can also be supported histopathologically and the overall pattern of histopathologic lesions may suggest a specific diagnosis. However, because of similar pathologic features seen in VHF and a variety of other viral, rickettsial, and bacterial infections, unequivocal diagnosis can only be made by laboratory tests such as immunohistochemistry and serology. The main pathological differential diagnosis should include viral hepatitis, leptospirosis, malaria, and rickettsial diseases.

CONFIRMATORY TESTING

Most of the viral HF can be diagnosed readily and rapidly from blood by a combination of antigen detection ELISA and IgM ELISA (Table 4). With most HF this combination of tests yields a diagnosis in virtually all patients within 24-48 hours of presentation; some Rift Valley fever and Crimean Congo HF patients, generally those with less severe disease, are an exception. Some patients may require RT-PCR for enhanced sensitivity in mild or early cases or for genotyping. Major practical obstacles to diagnosis are timely transport of properly collected samples to laboratories capable of performing rapid, definitive tests.

Saliva, urine, and feces yield lower or no virus but are worth testing in many cases because of the epidemiologic implications and to broaden the experience base. Effusions are often good sources of virus in Lassa fever.

In patients convalescing from disease, IgM by ELISA test may be present for weeks or a few months, but the duration has not been carefully defined for substantial cohorts of patients. Paired sera tested by IgG ELISA or the usual serological tests may also be useful.

Virus can generally be isolated from organs at necropsy. Hantaviruses are an exception possibly because of the general difficulty in their cultivation.

IHC and ISH tests of formalin-fixed tissues with specific antibodies and nucleic acid probes have proven to be sensitive and specific methods for confirmation of cause of HF. These methods have a unique role in cases in which archival tissues are the only specimens available for diagnostic testing. Electron microscopy may have limited usefulness in some instances such as with filovirus infections.

TREATMENT AND PREVENTION

The HF viruses may pose a potential for nosocomial transmission and may be exotic to the region where patients are hospitalized so that particular caution should be taken in patient care. The topic has been hotly debated but the biological basis for these concerns has been reviewed recently and guidelines for the US formulated.

Although there are no formal studies, there is a very strong clinical impression that VHF patients have a fragile vascular bed that responds poorly to being moved and so prompt, local hospitalization is desirable. The US military experience in Korea is instructive. During the 1950's when HFRS was common and usually diagnosed early the evacuation of troops to specialized facilities by helicopter was rapid and was a marked improvement over the prolonged, traumatic journey by jeep or ambulance. Later, the occasional patients were not recognized as early and evacuated long distances late in their course by airplane with a negative influence on their clinical course.

Supportive care for bleeding manifestations, fluid and electrolyte imbalance, shock, and hypoxia are much as recommended for any critically ill patients. These may be life saving for DHF/DSS patients and contribute to survival in other hemorrhagic fevers but probably do not overcome the overwhelming viral replication and tissue damage of, for example, an Ebola (Zaire subtype) patient. In general, fluid administration should be conservative because of the likelihood of precipitating pulmonary edema so that earlier use of cardiotonic and presser drugs is indicated. In HPS fluid management during hypotension or shock in the face of increased-permeability pulmonary edema is particularly difficult. Hypoxia may rapidly worsen in HPS and this should be planned while observing or transporting patients. The renal failure of HFRS and the severe hepatic involvement of yellow fever should be anticipated when dealing with these infections.

The antiviral drug ribavirin is effective against arenaviruses in vitro and in animal models. There is good evidence for its efficacy in human Lassa fever and such patients should receive intravenous ribavirin if their AST is >150 U/ml indicating a severe course. The New World arenavirus HF may also be responsive to the drug and it should be used in those cases. In the endemic zone, Argentine HF is routinely and successfully treated with adequate amounts of passive antibody infusion but ribavirin would be a reasonable alternative should convalescent plasma not be available.

Ribavirin has also proven effective in HF due to bunyavirid infections. Crimean-Congo HF may well respond to intravenous drug and ribavirin should be used in these cases. Rift Valley fever animal models respond and it would be rational to attempt therapy in human HF patients.

Vaccines are available for a few of the VHF and may be used to prevent disease. Other preventive measures are aimed at rodent and arthropod control and avoidance.

Table 1. Geography and epidemiology of HF viruses

VIRUS	DISEASE	GEOGRAPHY	VECTOR/RESERVOIR	HUMAN INFECTION
ARENAVIRIDAE				
Junin	Argentine HF	Argentine pampas	Small field rodent, <i>Calomys musculus</i> .	Infects agricultural workers disproportionately. Aerosol transmission to humans.
Machupo	Bolivian HF	Bolivia, Beni Province	Small field rodent, <i>C. callosus</i>	Rural residents and farmers main target; rodent can invade towns to cause epidemics. Aerosol transmission to humans.
Guanarito	Venezuelan HF	Venezuela, Portuguesa State	Chronic infection of field rodent, <i>Zygodontomys brevicauda</i>	Rural residents in newly developed area in Venezuela with small farms
Sabia	?	? rural area near Sao Paulo, Brazil	Presumably chronic infection of unidentified rodents	Single infection observed in nature: little information on potential
Lassa	Lassa fever	West Africa	Chronic infection of rodents of the genus <i>Mastomys</i> .	The reservoir rodent is very common in Africa and the disease is a major cause of severe febrile illness in West Africa. Spread to man occurs by aerosols and by capturing the rodent for consumption, as well as person-to-person transmission. Lassa fever is the most commonly exported HF.
BUNYAVIRIDAE				
Rift Valley fever	Rift Valley fever	Sub-Saharan Africa	Vertical infection of flood-water <i>Aedes</i> mosquitoes. Epidemics occur from horizontal transmission by many different mosquito species between domestic animals, particularly	Humans acquire by mosquito bite; contact with blood or offal of infected sheep, cattle, or goats; and aerosols generated from infected domestic animals. No inter-human transmission observed

			sheep and cattle.	
Crimean Congo HF	Crimean Congo HF	Africa, Middle East, Balkans, southern Soviet Union, western China	Tick-mammal-tick infection. Vertical infection occurs in ticks. <i>Hyalomma</i> ticks are thought to be the natural reservoir but other genera may become infected and transmit.	Tick bite; squashing ticks; and exposure to aerosols or fomites from slaughtered cattle and sheep. (Domestic animals do not evidence illness but may become infected when transported to market or when held in pens for slaughter.) Nosocomial epidemics observed on numerous occasions.
Hantaan, Seoul, Puumala , and others	Hemorrhagic fever with renal syndrome (HFRS)	World-wide, depending on rodent reservoir	Horizontal infection in a single rodent genus or species typical of the virus. Viruses associated with HFRS have been obtained from Muridae (subfamily Murinae) or from Avicolinae rodents	Aerosols from infected rodents. Some infections may be acquired from secondary aerosols or droplets from shed rodent excreta and secreta or from rodent bites. Interhuman transmission never documented.
Sin Nombre, Black Creek Canal, Bayou, and others	Hantavirus pulmonary syndrome (HPS)	Americas	As for hantaviruses causing HFRS. All viruses associated with HPS have come from Muridae (subfamily Sigmodontinae) rodents, if the reservoir is known.	As for hantaviruses causing HFRS. Entering abandoned, closed buildings may be a particular risk in some settings.
FILOVIRIDAE				
Marburg , Ebola	Marburg HF Ebola HF	Africa, ?Philippi nes	Unknown	Infection of index case occurs by unknown route. Later spread among human or non-human primates by close contact with another case. Aerosol transmission suspected in some monkey infections.
FLAVIRIDAE				
Yellow	Yellow	Africa, South	Mosquito-monkey-	Mosquito infection of humans

fever	fever	America	mosquito maintenance with occasional human infection when unvaccinated humans enter forest. Formerly large epidemics among humans with <i>Aedes aegypti</i> as mosquito vector.	entering forest and encountering infected sylvatic vector. Emergence of epidemics into African savannas using specific <i>Aedes</i> mosquito vectors. In cities or villages interhuman transmission by <i>Ae. aegypti</i> . Fully developed cases are no longer viremic and direct interhuman transmission not believed to be a problem although the virus is highly infectious (including aerosols) in the laboratory
Dengue (Types 1-4)	Dengue HF, dengue shock syndrome (DHF/DSS)	Tropics and sub-tropics world-wide	Maintained by <i>Aedes aegypti</i> -human- <i>Aedes aegypti</i> transmission with frequent geographic transport of virus.	DHF/DSS is a problem only in areas where multiple dengue viruses are being transmitted. With the increased world-wide distribution of <i>Ae aegypti</i> and movement of dengue viruses in travelers, this zone is enlarging. The disease was first noted in southeast Asia but is now common in the Americas and the Caribbean.
Kyasanur Forest disease (KFD)	KFD	Limited area of Mysore State, India	Tick-vertebrate-tick	Most infections occur from tick-bite acquired in rural areas of the endemic zone. Monkey die-offs may accompany increased virus activity.
Omsk HF (OHF)	OHF	Western Siberia	Poorly understood cycle involving ticks, voles, muskrats, and possibly water-borne transmission	Few cases in recent years.
Middle Eastern isolate		Middle East? Africa?	Unknown. Surmised to involve tick-domestic livestock-tick cycle by	Transmitted to humans working in livestock-related occupations by unknown route

			analogy to genetically related tick-borne flaviviruses	
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