Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

available at http://health.elsevier.com/tcmj

Tzu Chi Medical Journal

Review Article

The Role of Comparative Pathology in the Investigation of Zoonoses

Chen-Hsuan Liu[1], Yung-Hsiang Hsu[2]*

[1]*Department of Veterinary Medicine, National Taiwan University, Taipei, Taiwan*
[2]*Department of Pathology, Buddhist Tzu Chi General Hospital and Tzu Chi University, Hualien, Taiwan*

Article info

Article history:
Received: April 13, 2007
Revised: May 1, 2007
Accepted: May 8, 2007

Keywords:
Comparative pathology
One medicine
Zoonoses

Abstract

Emerging and re-emerging zoonoses have raised great concerns in both human and animal health worldwide in the past 20 years. Rudolph Virchow proposed a "one medicine" discipline and emphasized the importance of cooperation 150 years ago. In the face of emerging threats from unpredictable zoonoses, human medicine and veterinary medicine should not be separate and independent sciences. Anatomic pathologists who are capable of analyzing and interpreting anatomical manifestations of diseases to obtain a definite diagnosis or exclude a wide variety of diseases play an important role in the diagnostic team. Although disease-associated microbes are numerous, morphologic patterns of tissue reaction caused by microbes are limited. Therefore, the interactions between microbes and host determine the histological changes in the target tissues. The contributions of anatomic pathology, with its use of morphologic similarities and special techniques, are important in zoonosis diagnosis. This can be seen in retrospective case studies of recent zoonoses such as multinucleated syncytial giant cells in severe acute respiratory syndrome and mouse hepatitis virus infection, syncytial cells in *Henipahvirus* infection and paramyxovirus, neuronal vacuolation in bovine spongiform encephalopathy and variant Creutzfeldt-Jakob disease, and *Streptococcus suis* type 2 meningitis. In Taiwan, the Chinese Society for Comparative Pathology, which was established in 1994, provides for this interaction. Interlaboratory cooperation plays an important role in the diagnosis, surveillance, and control of emerging and re-emerging zoonoses. (*Tzu Chi Med J* 2007;19(3):127–133)

*Corresponding author. Department of Veterinary Medicine, National Taiwan University, 1, Section 4, Roosevelt Road, Taipei, Taiwan.
E-mail address: chhsuliu@ntu.edu.tw

1. Introduction

Emerging and re-emerging diseases have had a devastating impact on both human and animal health worldwide in the past 20 years, and zoonotic diseases have raised particular attention (Table 1). It is estimated that three-fourths of emerging diseases are zoonotic [1,2]. Facing the invasion of so many unpredictable pathogens and the emergence of bioterrorism as a global threat, a fast and definite diagnosis has become more important to eradicate or control the spread of diseases. Among diagnostic tools, anatomic pathology is effective in recognizing or excluding infectious diseases based on morphological changes in organs in affected species [3–5]. Human and veterinary pathologists are familiar with their own fields. However, German physician Rudolph Virchow (1821–1902), a towering figure in 19th medicine and pathology, stated, "Between animal and human medicine, there is no dividing line—nor should there be" while working on the zoonosis of trichinosis and hydatid disease [6,7]. This emphasis based on diseases across species lines established the concept of "one medicine" and led to the development of comparative medicine and comparative pathology.

The diagnosis of infectious diseases, especially zoonosis, requires the collaborative efforts of physicians, epidemiologists, microbiologists, parasitologists, and pathologists, as well as veterinarians to seek causal agents. Anatomic pathologists are capable of analyzing and interpreting anatomical or functional manifestations of diseases from autopsy, biopsy and cytology materials using routine processes and advanced diagnostic skills. They can provide important clues to the diagnostic team in characterization of diseases [3,8,9]. Years of experience have shown that data obtained from animal necropsies can be useful in determining the cause of death, which allows comparison with human counterpart diseases. Histopathological and cytopathological observations often allow for establishment of a definite diagnosis or exclusion of a wide variety of diseases. Compared with other diagnostic methods such as culture, immunodiagnosis and molecular biology, anatomic pathology is safe, economical, fast and reliable. It can help provide a disease diagnosis using formalin-fixed tissues, and direct visualization of lesions with fewer false-negative results.

2. Pathological diagnosis of infectious diseases

Although disease-associated microbes are numerous, morphologic patterns of tissue reaction caused by microbes are limited. Therefore, the interactions between microbes and host determine the histologic changes in the target tissues. This allows anatomic pathologists to analyze rare or unknown pathogens and quickly report their findings to a diagnostic team, which gathers information from the pathological, microbiological, immunological and molecular aspects to confirm disease. In pathological study, a routine hematoxylin and eosin (H&E) stained section can directly visualize some infectious agents or their products (e.g. bacterial clumps, most protozoans, all helminthes and viral inclusion bodies) within lesions (Table 2, Figs. 1–4) [10–12]. To detect infectious organisms not observed by routine H&E sections, advanced techniques in histochemistry, immunohistochemistry, transmission electron microscopy, polymerase chain reaction (PCR), *in situ* PCR, *in situ* hybridization, and other molecular techniques are being developed (Table 3, Figs. 5–10) [13–16].

3. Comparative pathology provides diagnostic clues in emerging and re-emerging zoonoses

It is very difficult to predict when and where the next zoonosis will emerge and the ultimate outcome depends on myriad interconnected factors. Thus, rapid and efficient collaboration based on the one medicine concept is necessary. Comparative pathology particularly focuses on comparison of the disease

Table 1 — Major emerging and re-emerging zoonoses in the last 20 years

Year	Disease	Main hosts
1986	Bovine spongiform encephalopathy (BSE), UK	Cattle, human
1993	Cryptosporidiosis, USA	Cattle, human
1994	New variant Creutzfeldt-Jakob disease (vCJD), UK	Human
1994	Hendra virus infection, Australia	Horse, human
1998	Nipah virus infection, Malaysia	Pig, human
1999	West Nile fever, USA	Bird, horse, human
2002	Severe acute respiratory syndrome (SARS), Asia	Human, Chinese horseshoe bat, masked palm civet, raccoon dog
2003	Highly pathogenic avian influenza (HPAI, avian flu), Asia	Bird, human
2003	Monkey pox, USA	Prairie dog, human
2005	*Streptococcus suis* type 2 infection, China	Pig, human

Table 2 — Spectrum of tissue reaction to infection (modified from *Robbins and Cotran, Pathologic Basis of Disease*, 7th edition)

Infectious agent	Tissue response	Example
Bacteria	Suppurative inflammation	*Streptococcus suis* type 2 infection (Fig. 1)
	Granulomatous inflammation	Tuberculosis
	Mononuclear inflammation	Leptospirosis
	Necrotizing inflammation	*Clostridium perfringens*
Fungi	Granulomatous inflammation	Histoplasmosis, blastomycosis
Helminthes	Granulomatous inflammation	*Toxocara* spp. migration
	Absence/minimal reaction	Trichinellosis (Fig. 1), *Toxocara* spp. migration
Prions	Vacuolation of neurons and neuropil	Scrapie (Fig. 2), bovine spongiform encephalopathy, variant Creutzfeldt-Jakob disease (Fig. 3)
Protozoans	Necrosis/granuloma	Toxoplasmosis, amebiasis, cryptosporidiosis
Viruses	Cytopathic	Herpesvirus
	Cytoproliferative	Papillomavirus, Epstein–Barr virus, Marek's disease
	Inclusion body	Adenovirus (intranuclear); poxvirus, rabies virus (intracytoplasmic) (Fig. 4); paramyxovirus, herpesvirus (intranuclear and intracytoplasmic)
	Syncytial formation	Paramyxovirus, herpesvirus, coronavirus, *Henipahvirus*

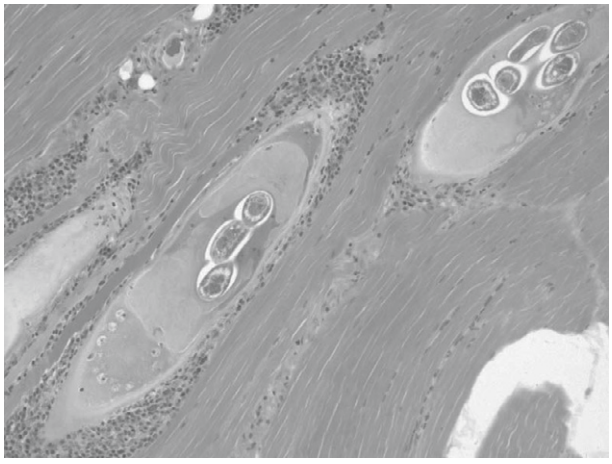

Fig. 1 — Pig trichinellosis: section shows larvae surrounded by nurse cells and mononuclear cell infiltration in the striated muscle (hematoxylin & eosin, 100×).

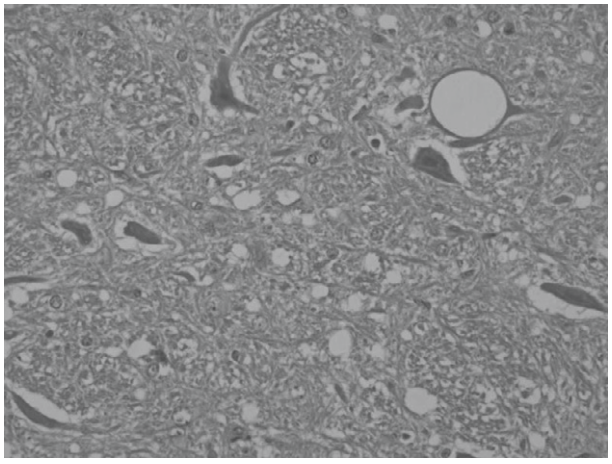

Fig. 2 — Sheep scrapie: prominent vacuolation in neurons and neuropil in the medulla oblongata (hematoxylin & eosin, 200×).

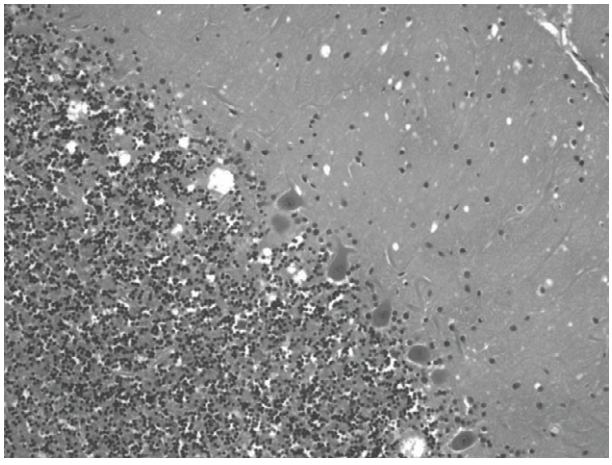

Fig. 3 — Human variant Creutzfeldt-Jakob disease: vacuolation in the cerebellar cortex and amyloid plaques (hematoxylin & eosin, 100×). [Courtesy of Dr Chin-Cheng Lee, Shin Kong Wu-Ho-Su Memorial Hospital, Taiwan.]

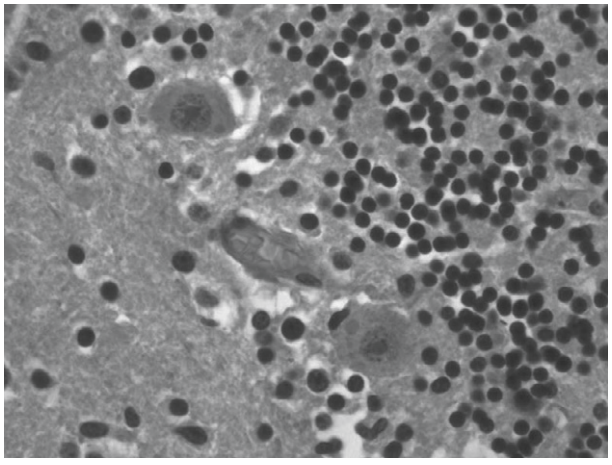

Fig. 4 — Human rabies: typical Negri body in the cytoplasm of the Purkinje cells of the cerebellum (hematoxylin & eosin, 200×).

Table 3 — Special techniques to detect infectious agents in tissues

Technique	Use
Acid-fast stain	Mycobacteria (Fig. 5), *Nocardia*, cryptosporidium
Brown–Brenn stain	Gram+, Gram–
Grocott's methenamine silver stain	Fungi
Immunohistochemistry	Antigen detection (Fig. 6)
In situ hybridization	Nucleic acid detection (Fig. 7)
In situ polymerase chain reaction	Nucleic acid detection
Mucicarmine stain	Cryptococci (Fig. 8)
Periodic acid-Schiff stain	Fungi (Fig. 9), amebae
Transmission electron microscopy	Ultrastructure of target cells or microbes (Fig. 10)

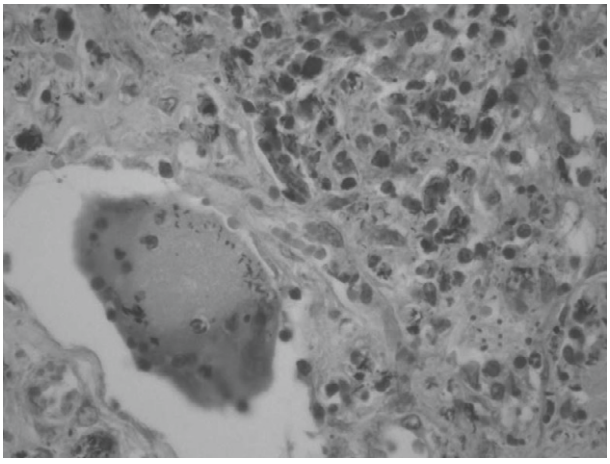

Fig. 5 — Bovine tuberculosis: many acid-fast bacilli, stained as red rods, are distributed in macrophages and multinucleated giant cells in the affected lung (acid-fast stain, 400×).

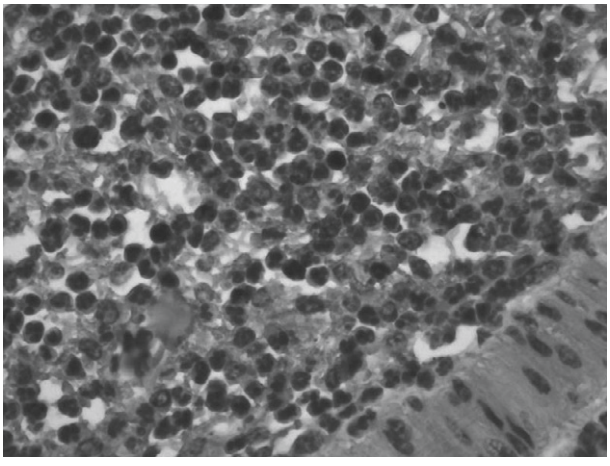

Fig. 6 — Pig streptococcal meningitis: the leptomeninges are heavily infiltrated by an admixture of neutrophils and lymphocytes. *Streptococcus suis* type 2 antigen is clearly demonstrated within the leukocytes by immunohistochemistry (non-biotin horseradish-peroxidase with hematoxylin counterstain, 400×).

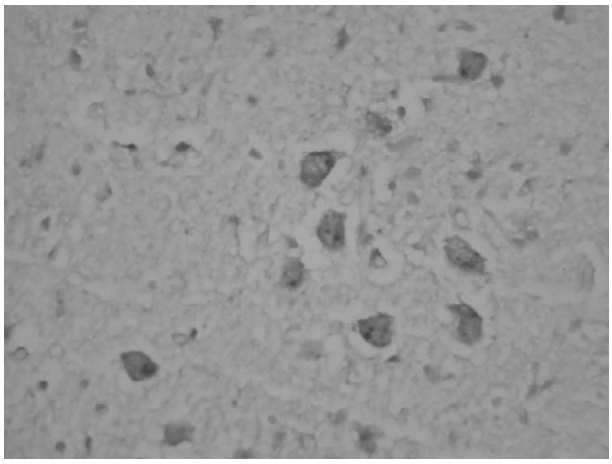

Fig. 7 — Horse rabies: positive signal is seen in affected neurons by *in situ* hybridization (200×). [Courtesy of Dr Lin-Lin Chueh, National Taiwan University, Taiwan.]

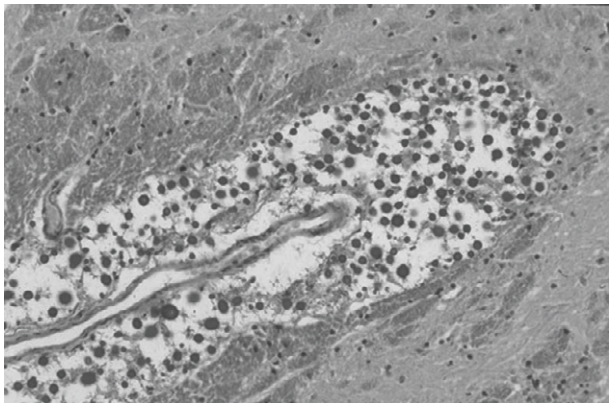

Fig. 8 — Mandrill cryptococcosis: a thick layer of meninges is infiltrated by numerous *Cryptococcus neoformans* organisms with large polysaccharide capsules (Mayer's mucicarmine, 200×).

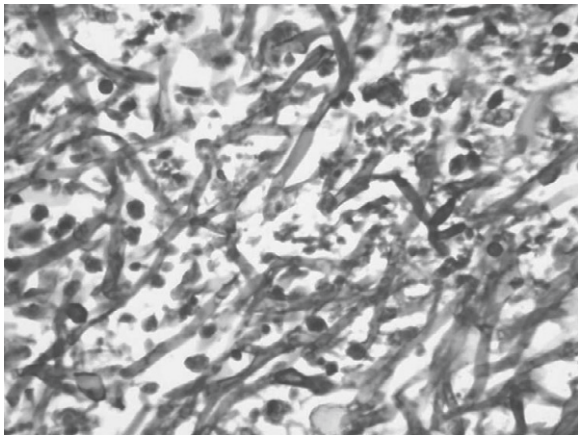

Fig. 9 — Human candidiasis: numerous *Candida* organisms with hyphae in the esophageal mucosa are demonstrated on periodic acid-Schiff stain (200×).

process in humans and other animals. To solve the complexities of emergence and re-emergence of zoonoses, effective and integrated collaboration between human and veterinary pathology is needed. With the use of morphologic features and auxiliary special techniques, anatomic pathologists contribute greatly to the diagnosis and consequent management of zoonoses (Table 4).

Retrospective case studies of recently occurring zoonoses reflect the importance of diagnostic pathology with its use of morphologic similarities in disease diagnosis. In 2003, an outbreak of severe acute respiratory syndrome (SARS) occurred in several countries and caused human deaths. The cause of the disease has been attributed to a coronavirus. Coronaviruses in animal species have been well known for years. In coronavirus mouse hepatitis virus (MHV), multinucleated syncytial giant cells (Fig. 11) in lesions of the liver and colon share common features with one of the characteristic lesions of SARS (Fig. 12) [16,17]. Further study indicated that intranasal infection of A/J mice with MHV strain 1 produced pulmonary pathologic features of SARS, including interstitial pneumonitis, dense macrophage infiltrates, giant cells, and hyaline membranes, resulting in death in all animals [17,18].

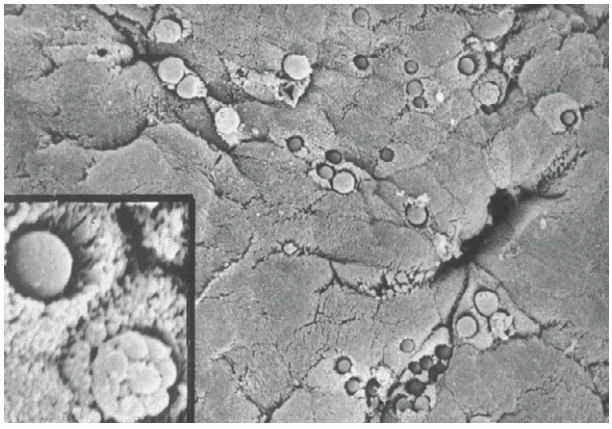

Fig. 10 — Pig cryptosporidiosis: numerous *Cryptosporidia* are embedded in the microvilli of the ileum (scanning electron microscope [SEM], 3750×). Insert: Opened schizont and unidentified stage of *Cryptosporidium* spp. surrounded by microvilli (SEM, 12,000×). [Reprinted with permission from: Liu CH, Chu RM, Lin YL. Intestinal cryptosporidiosis of pigs in Taiwan. *Chin J Gastroenterol* 1985;2:196–203.]

Outbreaks of equine Hendra virus infection in Australia in 1994 and Nipah virus infection in Malaysia in 1998 both caused mortalities in humans, horses and pigs. The two viruses are now classified as a new genus *Henipahvirus* in the family *Paramyxoviridae*. In past years, paramyxoviruses have caused devastating mortality in humans and major economic loss from animal outbreaks, such as that seen in measles in humans, canine distemper in canine species, and rinderpest in cattle, as well as Newcastle disease in poultry. The most commonly affected organs are those in the respiratory, gastrointestinal and nervous systems. Among the lesions, syncytial cells throughout the epithelium and in the vascular endothelium are a characteristic diagnostic feature [19].

Examples of scrapie in sheep versus kuru, and bovine spongiform encephalopathy in cattle versus variant Creutzfeldt-Jakob disease show common features of vacuolation of neuronal cell bodies and processes, spongiform changes in neuropil, no inflammatory infiltrates, and astrogliosis in the central nervous system [20]. Important morphologic observations from veterinary and human diagnostic pathologists provide valuable information in the exploration of mysterious diseases, such as prion diseases.

Streptococcus suis type 2 infection is a re-emerging zoonosis and is transmitted from pigs to humans through close contact. An infection in Sichuan, China, in 2005 caused 215 cases of human disease and 39 deaths. Purulent meningitis was the most common finding in both human and pig cases [21,22]. Routine H&E sections plus immunohistochemical staining provided rapid diagnosis and contributed greatly to the treatment of the disease.

Table 4 — Examples of common pathological features providing diagnostic clues of zoonoses

Causative agent	Human	Animal model	Common features
Coronavirus	Severe acute respiratory syndrome	Mouse hepatitis virus	Syncytial cells
Paramyxovirus	Hendra virus and *Henipahvirus* infection	Canine distemper, rinderpest, Newcastle disease	Syncytial cells in the epithelium and vascular endothelium
Prion	Kuru, variant Creutzfeldt-Jakob disease	Scrapie, bovine spongiform encephalopathy	Vacuolation of neuronal cell bodies and processes, spongiform change in neuropils, no inflammatory infiltrates, and astrogliosis
Streptococcus suis type 2	*Streptococcus suis* type 2 infection in humans	*Streptococcus suis* type 2 infection in pigs	Purulent meningitis
Leptospirosis	Leptospirosis	Leptospirosis	Interstitial nephritis
Cryptosporidium parvum	Cryptosporidiosis	Cryptosporidiosis	Organisms lining the epithelial cells
West Nile fever	West Nile fever	West Nile fever in horses	Nonsuppurative meningoencephalitis

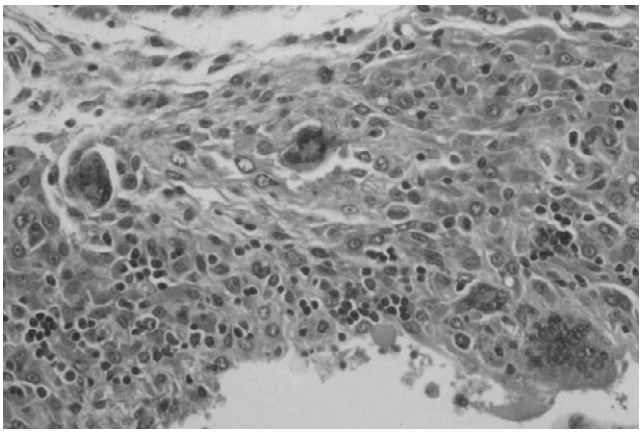

Fig. 11 — Mouse hepatitis virus: syncytial cells in the omentum (hematoxylin & eosin, 100×). [Courtesy of Dr Chung-Tiang Liang, National Laboratory Animal Center, Taiwan.]

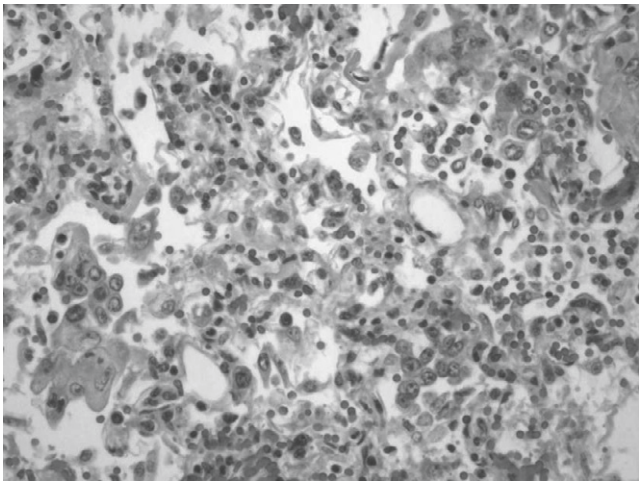

Fig. 12 — Severe acute respiratory syndrome (SARS): syncytial giant cells and hyaline membranes are observed in the lungs of SARS-infected patients (hematoxylin & eosin, 100×). [Courtesy of Dr Cheng-Hsiang Hsiao, National Taiwan University Hospital, Taiwan.]

4. Conclusion

Humans and other animals live in an unprecedented era in which emerging and re-emerging zoonoses can occur anywhere and anytime and the consequences can be severe. To control and prevent zoonotic diseases, human medicine and veterinary medicine should not be separate and independent sciences. Through the *one medicine* discipline, anatomic pathologists contribute valuable information to the diagnostic team by interpreting and analyzing morphologic changes. In Taiwan, this interaction was established through the Chinese Society for Comparative Pathology in 1994. In 2005, proceedings from the conferences of the society were published, and work continues in this area. We believe that comparative pathology will play an important role in the diagnosis, surveillance and control of unpredictable emerging and re-emerging zoonoses.

Acknowledgments

We are extremely grateful to Dr Chin-Cheng Lee of Shin Kong Wu-Ho-Su Memorial Hospital, Dr Cheng-Hsiang Hsiao of National Taiwan University Hospital, Dr Lin-Lin Chueh of National Taiwan University, and Dr Chung-Tiang Liang of the National Laboratory Animal Center for their kindness in providing the valuable photos in this article.

References

1. Gibbs EP. Emerging zoonotic epidemics in the interconnected global community. *Vet Rec* 2005;157:673–9.
2. MacKenzie WR, Hoxie NJ, Proctor ME, et al. A massive outbreak in Milwaukee of cryptosporidium infection transmitted through the public water supply. *N Engl J Med* 1994; 331:161–7.
3. Procop GW, Wilson M. Infectious disease pathology. *Clin Infec Dis* 2001;32:1589–601.
4. Schwartz DA, Bryan RT, Hughes JM. Pathology and emerging infections—quo vadimus? *Am J Pathol* 1995;147: 1525–33.
5. Schwartz DA, Herman CJ. The importance of the autopsy in emerging and reemerging infectious diseases. *Clin Infect Dis* 1996;23:248–54.
6. Brown C. Avian influenza: Virchow's reminder. *Am J Pathol* 2006;168:6–8.
7. Klauder JV. Interrelations of human and veterinary medicine; discussion of some aspects of comparative dermatology. *N Engl J Med* 1958;258:170–7.
8. Walker DH, Dumler JS. Will pathologists play as important a role in the future as they have in the past against the challenge of infectious diseases. *Infect Agents Dis* 1995;4:167–70.
9. Zaki SR, paddock CD. The emerging role of pathology in factious diseases. In: Scheld WM, Craig WA, eds. *Emerging Infections III.* Washington, DC: ASM Press, 1999:181–200.
10. Liu CH, Chu RM, Lin YL. Intestinal cryptosporidiosis of pigs in Taiwan. *Chin J Gastroenterol* 1985;2:196–203.
11. Matovelo JA, Landsverk T, Amaya Posada G. Cryptosporidiosis in Tanzanian goat kids: scanning and transmission electron microscopic observations. *Acta Vet Scand* 1984; 25:322–6.
12. McAdam AJ, Sharpe AH. Infectious diseases. In: Kumar VK, Abbas AK, eds. *Robbins and Cotran Pathologic Basis of Disease*, 7th edition. Philadelphia: Elsevier Saunders, 2004:343–414.
13. Aikawa M, Komata Y, Asai T, Midorikawa O. Transmission and scanning electron microscopy of host cell entry by *Toxoplasma gondii*. *Am J Pathol* 1977;87:285–96.
14. Boye M, Feenstra AA, Tegtmeier C, Andresen LO, Rasmussen SR, Bille-Hansen V. Detection of *Streptococcus*

suis by *in situ* hybridization, indirect immunofluorescence, and peroxidase-antiperoxidase assays in formalin-fixed, paraffin-embedded tissue sections from pigs. *J Vet Diagn Invest* 2000;12:224–32.
15. Liang CT, Chueh LL, Pang VF, et al. A non-biotin polymerized horseradish-peroxidase method for the immunohistochemical diagnosis of canine distemper. *J Comp Pathol* 2007;136:57–64.
16. Liang CT, Wu SC, Huang YT, et al. Immunohistochemical diagnosis of mouse hepatitis virus and *Mycoplasma pulmonis* infection with murine antiserum. *J Comp Pathol* 2004;131:214–20.
17. Tse GM, To KF, Chan PK, et al. Pulmonary pathological features in coronavirus associated with severe acute respiratory syndrome (SARS). *J Clin Pathol* 2004;57: 260–5.
18. De Albuquerque N, Baiq E, Ma X, et al. Murine hepatitis virus strain 1 produces a clinically relevant model of severe acute respiratory syndrome in A/J mice. *J Virol* 2006; 80:10382–94.
19. Maxie MG, Youssef S. Nervous system. In: Maxie MG, ed. *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals*, 7th edition. Philadelphia: Elsevier Saunders, 2007:281–457.
20. Lee CC, Liu CH, Wang CH. Pathology and molecular diagnosis of transmissible spongiform encephalopathy. *J Formos Med Assoc* 2001;5:562–8.
21. Higgins R, Gottschalk M. Streptococcal diseases. In: Straw BE, Zimmerman JJ, eds. *Diseases of Swine*, 9th edition. Oxford: Blackwell Publishing, 2006:769–83.
22. Tang J, Wang C, Feng Y, et al. Streptococcal toxic shock syndrome caused by *Streptococcus suis* serotype 2. *PLoS Med* 2006;3:e151.