Slov Vet Res 2022; 59 (4): 211–17
DOI 10.26873/SVR-1286-2022
UDC 599.742.28:591.2:616-091.5(513.1)

Case Report

PATHOLOGICAL FINDINGS IN AN OLD FEMALE GIANT PANDA – A CASE REPORT

Bangyuan Wu[1,2,3], Juan Wang[3], Tong Cai[3], Chengdong Wang[5], Desheng Li[5], Linhua Deng[5], Xi Peng[4]*

[1]Key Lab of Animal Ecology and Conservation Biology, Institute of Zoology, Chinese Academy of Sci-ences, 1-5 Beichenxi Road, Chaoyang, 100101 Beijing, [2]Key Laboratory of Southwest China Wildlife Re-sources Conservation (Ministry of Education), [3]College of Life Science, China West Normal University, Nanchong, 637009 Sichuan, [4]College of pharmacy, Chengdu University, Chengdu, 730000 Sichuan, [5]Key-laboratory of Endangered Animal Reproduction and Conservation and Genetics, China Conservation and Research Center for the Giant Panda, Wolong, 623006 Sichuan, China

*Corresponding author, E-mail: pengxi197313@163.com

Abstract: The giant panda (*Ailuropoda melanoleuca*) is one of the most endangered species in the world. Climate change and susceptibility to disease are two of the greatest threats to this species. We performed a necropsy and histopathological examination of the organs of an old panda and investigated the pathogenesis associated with death. Necropsy and histopathological observation revealed some typical age-related lesions, such as cataract, atherosclerosis, renal insufficiency and splenic atrophy. We also confirmed hepatic lesions associated with parasitic infection. Overall, our observations revealed that the predominant cause of mortality in this panda was multiple organ dysfunction (MOD).

Key words: aged; giant panda; multiple organ dysfunction; pathology

Introduction

The giant panda (*Ailuropoda melanoleuca*) is an enigmatic carnivore, adapted to a highly specialized ecological niche (1). To date, the phylogeny, demographic history, genetic variation, population structure and adaptive evolution of the giant panda have been extensively documented (2). However, wild giant pandas remain endangered and threatened by human interference, climate change, disease, and food shortages (1, 3). Although China established its first panda sanctuary in 1987, captive breeding, especially of old animals, is a major problem for captive giant pandas (4-6). Although there are pathological studies on geriatric diseases in humans and domestic animals (7-9), pathological lesions in old giant pandas have been reported only once (5). Therefore, in this study, we investigated pathological changes associated with mortality of a deceased geriatric giant panda. The results could make an important contribution to the limited literature in this field and help to improve the welfare of giant pandas in captivity.

Received: 25 January 2021
Accepted for publication: 28 November 2022

Materials and methods

History and observed clinical signs of the panda

At this stage, we examined the life history of the giant panda, including sex and age, living conditions, treatment situation and course of the giant panda. Pathological examination revealed

that the panda was in an emaciated state and died. In addition, clinical signs, mental status, nutritional status, fur, skin, eyes, visible mucous membranes and the condition of other body surfaces, and physiological indices were observed.

Necropsy

The body of the giant panda was thoroughly visually examined. After the external visual examination, a necropsy was immediately performed, which revealed changes in various organs. The organs with visible pathological lesions were photographed with a color video camera (Nikon 3 CCD) and further examined.

Histopathology

After thorough observation at a gross visual level, we selected tissue samples for histopathology from the lung, heart, aorta, liver, kidneys, spleen, digestive tract (including esophagus, duodenum, jejunum, ileum, colon, and rectum), mesenteric lymph nodes, ovaries, subcutaneous nodular lesion, and tissue from the decubitus. Tissue samples were then fixed in 4% paraformaldehyde (PFA) solution, dehydrated in a series of alcohols (at concentrations ranging from 70% to 100%) and embedded in paraffin wax. Tissue sections (5 µm) were prepared, stained with hematoxylin and eosin (H&E) and examined under a light microscope. Histological changes were photographed using a digital camera (Olympus, Japan).

Results

History and clinical signs

The case presented for necropsy involved a female giant panda, 15 years old, rescued from a nature reserve in China in 2005. Her age was estimated based on the degree of wear of the molars and skull growth (10). As she was unable to chew bamboo, she was fed a mixture of minced bamboo leaves and concentrated feed daily. Clinical examination revealed severe cataract (Fig. 1A), which indicated that the panda was suffering from anaemia and severe cardiac, hepatic and renal insufficiencies. After timely rescue and treatment, physiological values were essentially back to normal. In 2010, she went completely blind. Later, she gradually became emaciated and

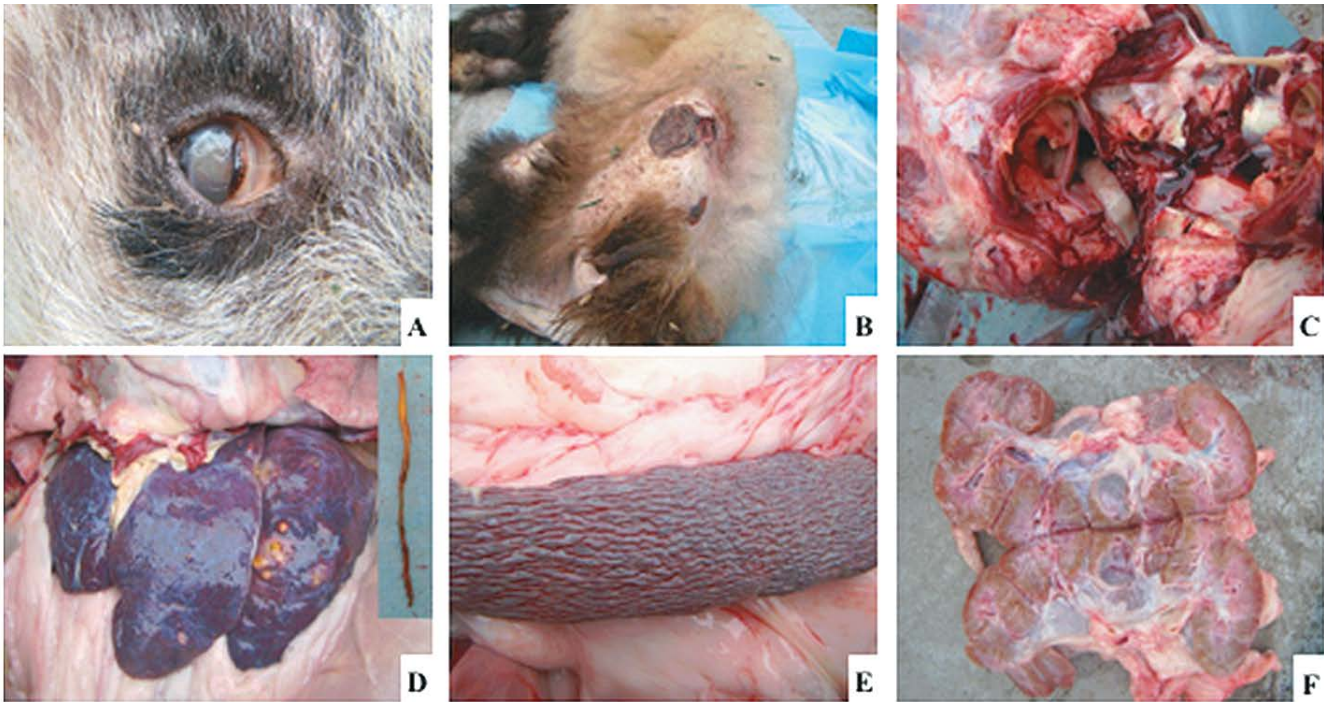

Figure 1: Gross pathological findings

A. A cataract in the right eye. B. A necrotic decubital tissue in the left gluteal region. C. Massive mucous secretion in the pharynx (arrow). D. Yellowish-white nodules on the liver surface and a nematode found in a nodule (arrow). E. The spleen shows a shrunken appearance (arrow). F. A urinoma in the left dilated kidney (arrow)

lost her fur, resulting in bald skin. In September 2013, decubital ulcers (Fig. 1B) appeared on the left buttock as a result of prolonged sleeping, which were difficult to treat. In late 2013, she fell into a deep coma and eventually died despite emergency rescue measures.

Necropsy findings

At necropsy, a large amount of viscous secretion was noted in the pharynx (Fig. 1C). The heart was enlarged and filled with blood clots. In addition, several pieces of a semitransparent jelly-like substance were seen adhering firmly to the aortic wall, showing signs of atherosclerosis. Multiple yellowish-white nodules of various sizes were observed on the surface of the liver, which contained caseous and purulent exudate and nematodes (Fig. 1D). The capsule of the spleen was contracted giving it a shrunken appearance (Fig. 1E). Both kidneys were swollen, and there were many yellowish-white mottled lesions on the surfaces. The left kidney had an enlarged pelvis filled with urine. (Fig. 1F). A single solid nodule (14×10×8 mm) was also found under the skin of the left abdomen.

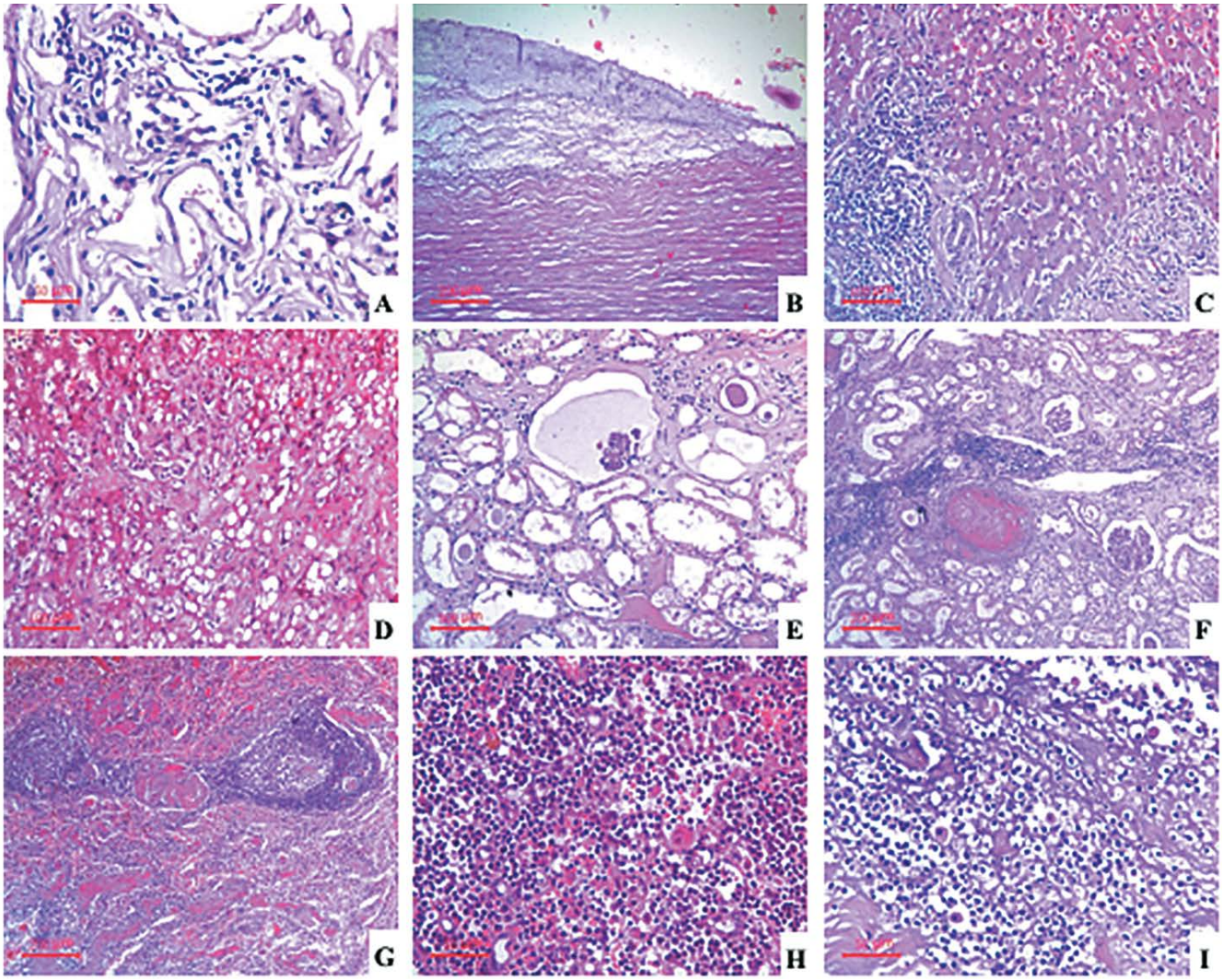

Figure 2: Histopathological changes, tissue sections stained with H&E

A. Lung. Lymphocyte infiltration and thickening of alveolar walls. B. Aorta. Atheromatous plaque. C. Liver. A proliferative nodule (square) and inflammatory cell infiltration (circle). D. Liver. Fatty degeneration (black arrow) and necrosis of hepato-cytes (blue arrow). E. Kidney. Glomerular atrophy (black arrow) and proteinaceous casts in the tubule lumina (blue arrow). F. Kidney. Hyperplasia of connective tissue (black arrow) and infiltration of inflammatory cells (blue arrow). G. Spleen. Age-related atrophy of the spleen (black arrow). H. Mesenteric lymph node. Lymphocytes (black arrow), macrophages (blue arrow), and erythrocytes (green arrow) in a lymphatic sinus. I. Decubital tissue. Numerous inflammatory cells infiltrating the necrotic muscle tissue of the decubital ulcer

Histopathological Findings

Histopathological examination of the lungs revealed slight thickening of the alveolar walls. This could be due to congestion and infiltration of inflammatory cells. Small scattered foci of inflammation were also observed, consisting mainly of lymphocytes and plasma cells, or macrophages phagocytosing black granules (Fig. 2A). In the aorta, an atheromatous plaque was noted that contained lipid-laden macrophages and proliferated connective tissue (Fig. 2B). In the liver, nodular cirrhosis with pseudohepatic lobules, fatty degeneration, and necrosis of hepatocytes was observed. Connective tissue hyperplasia, inflammatory cell infiltration, and a small focal abscess were also found in the examined tissue section of the liver (Fig. 2C and 2D). In the kidney, chronic sclerosing glomerulonephritis was diagnosed, characterized by glomerular atrophy, necrosis of the tubular epithelium, formation of proteinaceous casts, connective tissue hyperplasia, and infiltration of inflammatory cells (lymphocytes and neutrophils) (Figs. 2E and 2F). Hyperemia, abundant hemosiderin, and macrophages phagocytosing hemosiderin/erythrocytes were observed in the red pulp of the spleen (Fig. 2G). The splenic trabeculae were relatively enlarged due to age-related atrophy. Mild acute serous lymphadenitis was observed in the mesenteric lymph nodes, characterized by accumulation of lymph, fibrin, and inflammatory cells in the slightly enlarged sinuses of the lymph nodes (Fig. 2H). Only mild edema and mild exfoliation of the mucosal epithelium were noted in the digestive tract (images not shown). The single subcutaneous nodule was a benign fibroma composed of fibrocytes and desmocytes. The muscle tissue at the site of the pressure ulcer showed coagulation necrosis and inflammatory cell infiltration composed predominantly of neutrophils and macrophages (Fig. 2I).

Discussion

According to medical records, this female giant panda was about 23 years old when she died in 2013 after 8 years in captivity (11). At necropsy, massive pharyngeal mucous secretion was noted, possibly leading to the panda's asphyxiation and death. Previously, it was reported that difficulty in coughing up sputum in old pandas was due to decreased intrathoracic negative pressure, weakening of respiratory muscles and elastic retraction of the lungs (7). Lung function is known to deteriorate with age (12), and pulmonary alveolar epithelium permeability has been found to be higher in the elderly than in adults (13). It has been reported that age may play an important role in certain diseases, such as acute respiratory distress syndrome and chronic obstructive pulmonary disease (12), which is consistent with our findings in this report. In addition, some studies suggest that the lung plays an important role in the development of multiple organ dysfunction (MOD) (14, 15). MOD is more commonly reported after trauma and is associated with high mortality (16, 17). It has also been reported that the elderly are more susceptible and at higher risk of MODS (14). MOD is defined as a group of various chronic diseases, including chronic obstructive pulmonary disease and idiopathic pulmonary fibrosis (7), decreased glomeruli and tubulointerstitial fibrosis in the kidney (8), immune dysfunction and degeneration of the spleen(18), chronic heart disease (19), presbycusis (20), cataracts, and cognitive impairment (3). In our case of old giant panda, several chronic pathological changes such as cataracts, lung and kidney lesions, adipose tissue atrophy, atheromatous plaques in the aorta, and splenic atrophy were observed and reported, which developed along with parasitic infection of the liver and consequent emaciation. Histopathologically, the main findings that could be age-related were atheromatous plaques, reduction of renal glomeruli, age-related atrophy of the spleen, thickening of alveolar septa in the lungs, and connective tissue hyperplasia in the liver and kidney. These lesions were consistent with the pathological manifestations of ageing described in humans and other mammals, including giant pandas (5, 7, 8). A typical age-related change, "cataract", was due to the gradual loss of elasticity of the lens (21, 22). In addition, oxidised low-density lipoprotein (ox-LDL) has been reported to lead to endothelial dysfunction, which is considered to be an initial step in the formation of atheroma (23, 24). Ox-LDL has been shown to play an important role in the formation of lipid-laden macrophages, the primary cellular component of atherosclerotic lipid lesions (25). This may be the reason why we found the atheromatous aortic plaque in this case. We believe that the decrease in renal glomeruli in this

giant panda case is related to tubular necrosis and fibrosis, similar to some previous reports (26, 27). Age-related changes are well-known factors that influence the susceptibility to disease of almost all vital organs. The elderly individuals often show a markedly exaggerated host immune response to inflammatory stimuli (14). In the present study, the pathological lesions associated with inflammation, particularly the infiltration of inflammatory cells in the lung, kidney, liver, and lymph nodes, were possibly due to the spread of inflammation from bedsores or parasitic hepatitis to these organs. In general, systemic inflammatory response syndrome (SIRS) has been shown to have an adaptive survival function for the host, but in critically ill patients, uncontrolled production of inflammatory mediators can lead to MODS (28). The results of this study were consistent with the "2-hit" hypothesis in the development of MODS, which states that an initial insult primes the host such that a subsequent impairment, such as infection or surgery, greatly enhances the host response (18). We believe that in our case a combination of age-related pathological lesions and chronic parasitic infection led to MOD, and that MOD was the main cause of death in this old giant panda. The parasitic hepatitis could be the initial impairment, and the decubitus ulcer could be the subsequent impairment leading to increased damage, as the aging can increase susceptibility to organ dysfunction and systemic inflammation, as shown by previous reports (29, 30). The macroscopic and microscopic lesions in the liver were consistent with chronic verminous hepatitis, and it could be hypothesised that this giant panda already suffered from a parasitic infection such as the nematode *Baylisascaris schroederi* in the wild (31). In an anatomical study of 33 wild giant pandas, a 100% lumbricoid infection rate indicated the prevalence of parasitic infections in wild animals (32). A study examining causes of death in wild giant pandas from 1971 to 2005 found that the greatest threat to wild giant pandas is migration of visceral larvae (33). Consistent with the anatomical location of the nematode, the lesions found in the liver and the life cycle of the parasite (34), the parasite may have migrated from the bile duct to the liver. Currently, research in China mainly focuses on parasite species and associated morbidity in wildlife. However, in the future, more attention should also be paid to the transmission and control strategies of parasitic wildlife diseases to increase the overall life expectancy of wildlife. In addition, many infectious diseases of humans have hosts or vectors in animals, which places greater demands on research and control of animal diseases (35).

In conclusion, we believe that MOD was the main reason for the death of this old female giant panda. A series of age-related pathological lesions, supported by pre-existing pathological conditions such as liver dysfunction due to parasite infestation, eventually led to poor physical condition, emaciation, respiratory failure and death.

Acknowledgments

Authors wish to express gratitude to China Conservation and Research Center for the Giant Panda for their cooperation and assistance in conducting the study.

Supported by the program for the Education Department of Sichuan Province (project no. 17ZB0425), the Meritocracy Research Funds of China West Normal University (project no. 17YC349) and the Fundamental Research Funds of China West Normal University (project no. 20A003).

The datasets supporting the results of this document are contained within the article. Any additional data may be requested to the corresponding author.

The datasets supporting the results of this document are contained within the article. Any additional data may be requested to the corresponding author.

The authors declare no conflict of interest.

Conceptualization and supervision: W.B.Y. and P.X., Methodology, investigation, data curation, W.B.Y., W.J., C.T., W.C.D., L.D.S., D.L.H, writing-original draft preparation: W.B.Y. and W.J., writing–review and editing: P.X. All authors have read and agreed to the published version of the manuscript.

References

1. Lu Z, Warren EJ, Marilyn MR, et al. Patterns of genetic diversity in remaining giant panda populations. Conserv Biol 2001; 15(6): 1596–607.

2. Wei FW, Yibo H, Lifeng Z, Michael WB, Zan XJ, Lei Z. Black and white and read all over: the

past, present and future of giant panda genetics. Mol Ecol 2012; 21(23): 5660–74.

3. Steinmeyer C, Pennings PS, Foitzik S. Multicolonial population structure and nestmate recognition in an extremely dense population of the European ant *Lasius flavus*. Insect Soc 2012; 59(4): 499–510.

4. Burrell C, Hemin Z, Desheng L, Chengdong W, Caiwu L, Aitken-Palmer C. Hematology, serum biochemistry, and urinalysis value in the giant panda (*Ailuropoda melanoleuca*). J Zoo Wildlife Med 2017; 48(4): 1072–6.

5. Guo DZ, Shiqi Z, Jiakui L, Xueying H, Shijin Y. Pathology observation on multiple organ faliure of aging giant panda. Chinese J Anim Vet Sci 2002; 33(3): 295–8.

6. Liu DZ, Guiquan Z, Rongping W, Hemin Z, Jiming F, Ruyong S. Effects of sex and age on the behavior of captive giant pandas (*Ailuropoda melanoleuca*). Acta Zool Sin 2012; 48(5): 585–90.

7. Britto RR, Zampa CC, de Oliveira TA, Prado LF, Parreira VF. Effects of the aging process on respiratory function. Gerontology 2009; 55(5): 505–10.

8. Weinstein JR, Sharon A. The aging kidney: physiological changes. Adv Chronic Kidney Dis 2010; 17(4): 302–7.

9. Föllmi J, Steiger A, Walzer C, et al. A scoring system to evaluate physical condition and quality of life in geriatric zoo mammals. Anim Welfare 2007; 16(3): 309–18.

10. Wei FW, Jinchu H, Guangzan X, Mingdao J, Qitao D, Zhaomin Z. The age of determination for giant panda. Acta Theriol Sin 1989; 8(3): 161–5.

11. Zhou X, Yan H, Jingyan H, Shiqiang Z, Dian L. Behavioral development of gaint panda and influencing factors in husbandry management. J Heilongjiang Univ Sci Technol 2013; 34(2): 106–10.

12. Sevransky JE, Haponik EF. Respiratory failure in elderly patients. Clin Geriatr Med 2003; 19(1): 205–24.

13. Yang MF, He ZX, Wang SW. Evaluation of pulmonary epithelial permeability with 99Tc-m-DTPA radioaerosol. Chin J Nucl Med 2002; 22(4): 250–2.

14. Wang XP, Zhu QL, Xue Q, et al. Role of the lung in the progression of multiple organ dysfunction syndrome in ageing rat model. Chin Med J 2012; 125(015): 2708–13.

15. Wang SW, Han YL, Qian XS, et al. Clinical features of multiple organ failure in the elderly: a report of 1605 cases. Chin J Mult Organ Dis Elderly 2002; 1(1): 7–10.

16. Durham RM, Moran JJ, Mazuski JE, Shapiro MJ, Baue AE, Flint LM. Multiple organ failure in trauma patients. J Trauma Acute Care 2003; 55(4): 608–16.

17. Goris RJA. Pathophysiology of multi-organ failure: an overview. Clin Intensive Care 1991; 2(6): 5–15.

18. Zhu H, Pin Y, Yanfeng X, et al. Structual and functional changes of immune system in natural aged SD rats. Acta Laborator Anim Sci Sin 2018; 26(1): 95–100.

19. Stewart S, MacIntyre K, Capewell S, McMurray JJV. Heart failure and the aging population: an increasing burden in the 21st century? Heart 2003; 89(1): 49–53.

20. van Boxtel MV, Buntinx F, Houx P, Metsemakers J, Knottnerus A, Jolles J. The relation between morbidity and cognitive performance in a normal aging population. J Gerontol A Biol 1998; 53(2): 147–54.

21. Truscott RJW. Eye lens proteins and cataracts. In: Uversky VN, Fink AL, eds. Protein misfolding, aggregation, and conformational diseases. Boston : Springer, 2006: 435–47. (Protein Reviews, vol. 6) doi: 10.1007/978-0-387-36534-3_21

22. Olcaysu OO, Kivanc SA, Altun A, Cinici E, Altinkaynak H and Ceylan E. Causes of disability, low vision and blindness in old age. Turk J Geriatr 2014; 17(1): 44–9.

23. Cao C, Qi Y, Chen W, Zhu Y and Chen X. Effects of IKK on oxidised low-density lipoprotein-induced injury in vascular endothelial cells. Heart Lung Circ 2013; 22(5): 366–72.

24. Toth PP. Low-density lipoprotein reduction in high-risk patients: how low do you go?. Curr Atheroscler Rep 2004; 6(5): 348–52.

25. Derian CK, Lewis DF. Activation of 15-lipoxygenase by low density lipoprotein in vascular endothelial cells. Relationship to the oxidative modification of low density lipoprotein. Prostag Leukotr ESS 1992; 45(1): 49–57.

26. Zhou C, Wu J, Torres L, et al. Blockade of osteopontin inhibits glomerular fibrosis in a model of anti-glomerular basement membrane glomerulonephritis. Am J Nephrol 2010; 32(4): 324–31.

27. Kanbay M, Kasapoglu B, Perazella MA. Acute tubular necrosis and pre-renal acute kidney injury: utility of urine microscopy in their evaluation-a systematic review. Int Urol Nephrol 2010; 42(2): 425–33.

28. Nathens AB, Marshall JC. Sepsis, SIRS, and MODS: What's in a name? World J Surg 1999; 20(4): 386–91.

29. Nin N, Lorente JA, De Paula M, et al. Aging increases the susceptibility to injurious mechanical ventilation. Intensive Care Med 2008; 34(5): 923–31.

30. Eachempati SR, Hydo LJ, Shou J, Barie PS. Outcomes of acute respiratory distress syndrome (ARDS) in elderly patients. J Trauma 2007; 63(2): 344–50.

31. Zhou X, Yu H, Wang N, Xie Y, Yang GY. Molecular diagnosis of *Baylisascaris schroederi* infections in giant panda *(Ailuropoda melanoleuca*) feces using PCR. J Wildl Dis 2013; 49(4): 1052–5.

32. Feng WH, Anju Z, eds. Giant panda reproduction and disease research. Chengdu, Sichuan, China : Sichuan Science and Technology Press, 1991: 244–8.

33. Zhang JS, Peter D, Huali H, Guangyou Y, A Marm K, Shuyi Z. Parasite threat to panda conservation. EcoHealth 2007; 5(1): 6–9.

34. Blouin MS, Liu J, Berry RE. Life cycle variation and the genetic structure of nematode populations. Heredity 1999; 83(3): 253–9.

35. Kaupke A, Rzezutka A. Epidemiology of the invasion of *Cryptosporidium parvum* in farm and wild animals. Med Weter 2017; 73(7): 387–94.

PATOLOŠKE UGOTOVITVE PRI SAMICI VELIKEGA PANDE – POROČILO O PRIMERU

B. Wu, J. Wang, T. Cai, C. Wang, D. Li, L. Deng, X. Peng

Izvleček: Veliki panda (*Ailuropoda melanoleuca*) je ena najbolj ogroženih vrst na svetu. Vrsto najbolj ogrožajo podnebne spremembe in dovzetnost za bolezni. Opravili smo nekropsijo in histopatološki pregled organov starega pande ter raziskali patogenezo, povezano s smrtjo. Z nekropsijo in histopatološkim opazovanjem smo odkrili nekatere značilne starostne spremembe, kot so katarakta, ateroskleroza, ledvična insuficienca in atrofija vranice. Potrdili smo tudi jetrne spremembe, povezane s parazitsko okužbo. Naša opažanja so pokazala, da je bil prevladujoči vzrok smrti tega pande disfunkcija več organov (MOD).

Ključne besede: star; veliki panda; disfunkcija več organov; patologija