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Jurnal Veterinar Malaysia is the official journal of the Veterinary Association Malaysia (VAM). It was published formally as *Kajian Veterinar* and the Malaysian Veterinary Journal.

Researcher papers on various aspects of veterinary medicine, animal science and research are invited for publication either as full articles or short communication. Review papers and abstracts of articles of local interest are also published from time to time. The publisher (VAM) does not hold itself responsible for statements made in the journal by contributors. Unless so stated, materials in the Journal do not reflect an endorsement or an official attitude of VAM or the Editorial Board of JVM.

THE VETERINARY ASSOCIATION MALAYSIA
ISSN 9128-2506

**INTERSTITIAL PULMONARY FIBROSIS IN A MALAYSIAN CAPTIVE ASIAN ELEPHANT
(*ELEPHAS MAXIMUS*)**

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SUMMARY

A 14-year-old female captive Asian elephant in a Malaysian zoo with a history of positive sero-reactivity to Elephant TB STAT-PAK assay was humanely euthanized due to chronic weight loss and poor response to treatment. Postmortem revealed generalized congestion and emphysematous lungs. Microscopically, there were severe pulmonary emphysema, eosinophilic hyaline membrane and infiltration of mature fibrocytes in the alveolar walls. Based on the histopathological findings interstitial pulmonary fibrosis was diagnosed. The possible cause of which although not completely known, is most likely due to hypersensitivity. The allergic reaction could have been caused by exposure to antigens of avian origin or the *Mycobacterium gilvum*, a non-tuberculous mycobacterium (NTM), isolated from the lung. This is the second case reported in elephants globally, though it has been well studied in humans and reported in horses. Providing good husbandry for elephants in captivity is important to reduce the occurrence of such condition.

Key Words: Hypersensitivity pneumonitis, NTM, bird antigen, Asian elephant

INTRODUCTION

Asian elephants (*Elephas maximus*) are the largest terrestrial mammal in Asia and the only living species of the genus *Elephas* found in Southeast Asia (Shoshani and Eisenberg, 1982). With a gradually declining population, Asian elephants have been listed as endangered by the International Union for the Conservation of Nature (IUCN) over two decades ago (Choudhury *et al.* 2008). The survival and conservation of captive elephants has been threatened by diseases and husbandry problems worldwide. Captive elephants were found to die at a much younger age and are declining in numbers due to low birth and high mortality rates (Sukumar, 2003).

Interstitial pulmonary fibrosis due to hypersensitivity pneumonitis is well studied in humans and has historically been associated with occupational exposure to allergens (Blatman and Grammer, 2012). The disease has also been reported to be life-threatening in horses resulting in respiratory impairment and death (Dungworth, 1982). Very little is known about interstitial pulmonary fibrosis in elephants with only one reported case in an Africa elephant globally (Johnson *et al.*, 1986). The condition is caused by multiple etiologies such as hypersensitivity pneumonitis, pneumotoxins, microbes, silica dust and asbestos; characterized by inflammation of the alveoli walls and subsequent formation of excess fibrous connective tissue (Sweeney *et al.*, 2002). Clinical diagnosis of hypersensitivity pneumonitis is challenging and often times misdiagnosed for bacterial or viral pneumonia (Moore *et al.*, 2004). The numerous expensive laboratory tests involved and insufficient information on

immunologic disorders in elephants may contribute to the under-reporting of the disease. This paper presents a case of interstitial pulmonary fibrosis in a captive Asian elephant and highlights the importance of providing good husbandry practices to reduce its occurrence.

HISTORY

During a cross-sectional study to determine the seroprevalence of tuberculosis in captive Asian elephants in Peninsular Malaysia undertaken by Universiti Putra Malaysia (UPM), a 14-year-old 1500 kg female elephant was found to show positive sero-reactivity to Elephant TB Stat-Pak Assay (ChemBio, USA) in January 2012. Subsequent trunk washes collected were PCR negative for *Mycobacterium tuberculosis* (*M. tuberculosis*) using the AmpliSens MBT-EPh PCR kit (AmpliSens Biotechnologies) and culture negative for *M. tuberculosis* on Lowenstein-Jensen (LJ) media (Oxoid, UK®) at that time. The elephant was housed with seven other elephants in an open enclosure with perimeter fencing away from visitors. They were fed daily with freshly cut banana trunk, rock melon, sweet potato, Napier grass, sugarcane and concentrates. The feed was accessible to wild birds and it was observed that many trees planted in the vicinity of the elephant enclosure provided good roosting sites for many wild birds.

In July 2012, the elephant was inappetent, losing weight and showed respiratory problem. A series of palliative treatments were administered (trimethoprim-sulphadiazine 22 mg/kg, flunixin meglumine 2 mg/kg, enrofloxacin 5 mg/kg, selenium 50 ml, B-complex 50 ml and multivitamin 50 ml) but her condition continued to deteriorate. By September 2012, the body score was 5/11 (emaciated) based on the Asian elephant body condition

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index (Wemmer *et al.*, 2006), the elephant became recumbent for several days and euthanasia was proposed upon humanitarian grounds. Blood and trunk washes were taken before euthanasia for haematology and clinical chemistry profile, and bacteriology respectively.

POSTMORTEM EXAMINATION

During postmortem, precautionary measures were taken by wearing N95 facemask and the opening the thoracic cavity was kept last since the elephant had a history of positive sero-reactivity to Elephant TB Stat-Pak Assay. Postmortem examination revealed generalised pulmonary congestion, oedema and emphysema of lungs but the mucosal linings of the elephant trunk, trachea and bronchi were normal. There were no significant findings in the heart, liver, kidney, spleen, lymph nodes, ovaries and gastrointestinal tracts.

Tissue samples were taken aseptically from the lung, liver, heart, spleen, tonsils, kidneys and lymph nodes (axillary, apical, bronchial, sternal, mesenteric and inguinal) for microbiology. Tissues for histopathology were fixed in 4% buffered formalin and stained with Hematoxylin and Eosin (H & E). For microbiology, the trunk washes were first decontaminated with 2% NaOH before cultured onto Lowenstein-Jensen (LJ) media and blood agar (Oxoid, UK[®]), and incubated at 37°C. No growth was observed after four weeks of incubation on LJ media and 48 h of incubation on blood agar. Microscopy examination of the tissues collected for bacteriology revealed few Gram positive cocci in the axillary lymph node and Gram negative small rods in the tonsils. No bacteria were seen in the liver, kidneys, heart and sternal, mesenteric and inguinal lymph nodes. The tissue

specimens were cultured onto blood agar (Oxoid, UK[®]) and MacConkey agar (Oxoid, UK[®]), incubated at 37°C for at least 48 h, and on LJ media incubated at 37°C for 4 weeks. The spleen, tonsils, lungs and all lymph nodes (axillary, bronchial, apical, mesenteric and inguinal) yielded bacterial growths on blood agar which were identified using Gram staining and conventional biochemical tests (Table 1). Only the apical lobe of the right lung yielded yellowish colonies on LJ media after 10 days of incubation (Table 1). The organism was Zeihl-Neelsen acid-fast positive bacilli, and was identified as *Mycobacterium gilvum* by *hsp65* gene PCR amplification, sequencing and subsequent identification using the Basic Local Alignment Search Tool (BLAST) in the Gene Bank. Parasitology examination did not reveal any adult helminths in the gastrointestinal tract, and no ova, oocysts or larvae were detected in the faecal samples. Haematology results were normal but serum biochemistry revealed elevated liver enzymes including alanine aminotransferase (ALT) 41.2 (reference value: 1.5-3.0 U/L), aspartate aminotransferase (AST) 294.7 (reference value: 15-35 U/L), and creatine phosphokinase (CPK) 1050 (reference value: 50-250 U/L) (Duncan *et al.*, 1994). However, the laboratory does not have the test to determine the histamine level in the blood.

Histopathological findings revealed severe pulmonary emphysema with expansion of the alveolar space (Figure 1). There were eosinophilic hyaline membrane structures lining the alveolar wall (Figure 2). There was also infiltration of the alveolar wall by mature fibrocytes (Figure 3). Based on the microscopic findings, interstitial pulmonary fibrosis was diagnosed possibly due to hypersensitivity pneumonitis.

Table 1. Organisms isolated from bacterial culture of visceral organs and lymph nodes of *Elephas maximus*. Samples were cultured on Blood and MacConkey agar, and Lowenstein-Jensen media

Samples collected	Organisms isolated					
	<i>Staphylococcus pseudointermedius</i>	<i>Corynebacterium kutscheri</i>	<i>Streptococcus bovis-Streptococcus equines complex</i>	<i>Acinetobacter iwoffi</i>	<i>Pseudomonas aeruginosa</i>	<i>Mycobacterium gilvum</i>
Liver	-	-	-	-	-	-
Kidney	-	-	-	-	-	-
Heart	-	-	-	-	-	-
Spleen	-	-	+	+	-	-
Tonsils	-	-	+	-	+	-
Lungs	-	-	+	-	-	+
Sternal L/N	-	-	-	-	-	-
Axillary L/N	+	+	+	-	-	-
Bronchial L/N	-	-	+	-	-	-
Apical L/N	-	-	+	-	-	-
Mesenteric L/N	-	-	-	-	-	-
Inguinal L/N	-	-	-	-	-	-
Trunk wash	-	-	+	-	+	-

Legends; +, culture positive; -, culture negative; L/N, lymph node.

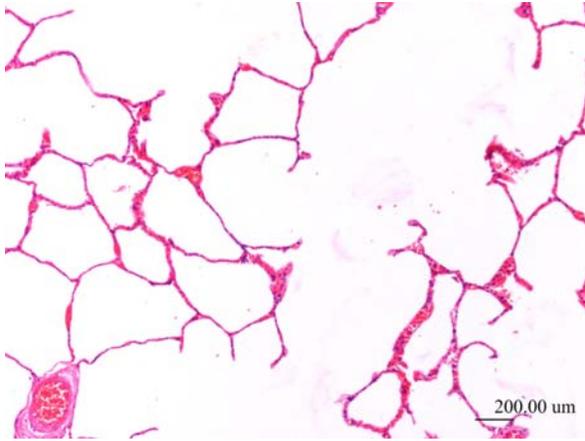


Figure 1. Pulmonary emphysema with expansion of alveolar space. (Hematoxylin and Eosin stain)

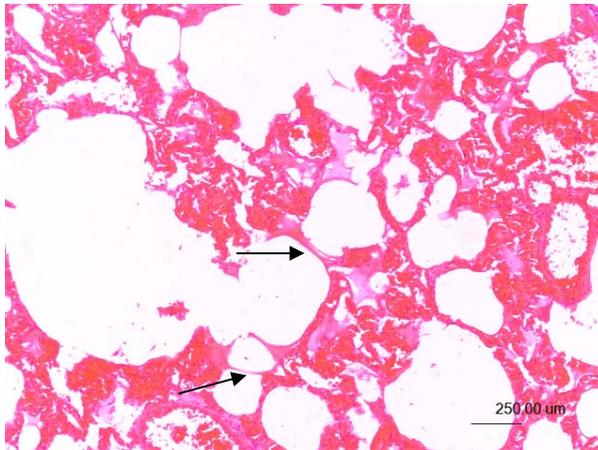


Figure 2. Eosinophilic hyaline membrane structures lining the alveolar wall (arrows). (Hematoxylin and Eosin stain)

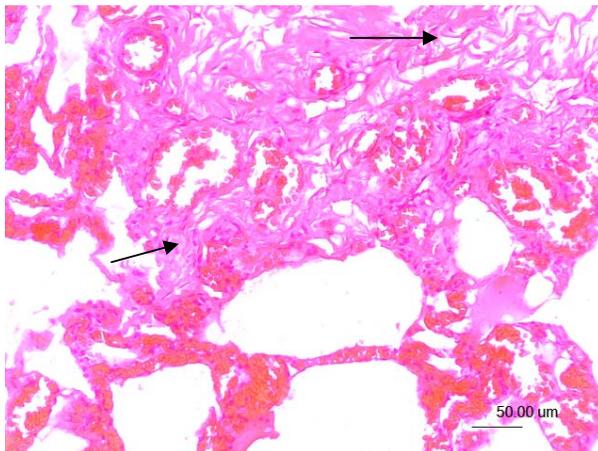


Figure 3. Infiltration of the alveolar wall by mature fibrocytes (arrows). (Hematoxylin and Eosin stain)

DISCUSSION

Hypersensitivity pneumonitis also known as extrinsic allergic alveolitis or *farmers' lung* is a well-documented condition in humans associated with chronic low level exposure to environmental allergens such as fungal spore and organic dusts (Bernstein *et al.*, 1985; Moore *et al.*, 2004; Kurup *et al.*, 2006). It is a diffused interstitial lung disease due to combined type III and type IV hypersensitivity reactions as a result of cell-mediated immunologic response to a variety of inhaled organic antigens (Agache and Rogozea, 2013). Similar condition called *hay sickness* in horses has been reported to be associated with exposure to allergic substances such as silica dust, asbestos, pneumotoxic chemicals and pyrrolizidine alkaloids (Asmundsson, 1983). The only documented case of interstitial pulmonary fibrosis in elephant was in an African elephant suspected to be exposed to thermophilic molds or bird antigens (Johnson *et al.*, 1986).

The elephant in this study might have got exposed to antigens of avian origin such as excreta or feathers of wild birds in contaminated feed or environment since many wild birds were seen roosting on trees within the vicinity of the elephant enclosure. Prolonged exposure to allergens could have triggered hypersensitivity pneumonitis which ultimately led to interstitial pulmonary fibrosis. Histopathology findings such as the absence of cellular infiltration are further suggestive of an allergic response. The hyaline degeneration of the alveoli and fibrous tissue deposition interferes with oxygen diffusion into the alveoli blood capillaries resulting in low amount of oxygen in the blood and insufficient oxygenated blood supply to body tissues. As the respiratory impairment affects the brain and other vital organs, the elephant became weak and finally recumbent. Damage to the sciatic nerve or its peroneal branch might have occurred as a result of prolonged recumbency, which led to pressure myopathy as indicated by the elevated levels of liver enzymes such as ALT, AST and CPK.

The bacterial organisms isolated from the postmortem tissues are not of clinical significance as they are normal gastrointestinal tract and environmental bacteria. The NTM isolated from the lung, *M. gilvum*, has never been reported to cause disease in humans or elephants. These organisms are saprophytes and are ubiquitous in soil and water (Wayne and Sramek, 1992). Elephants enjoy water and dust baths and are constantly exposed to these organisms. The potential pathogenicity of this organism cannot be underestimated as NTM have been reported to cause hypersensitivity pneumonitis in humans (Weiss and Glassroth, 2012). The possible cause of the interstitial pulmonary fibrosis in this case although not completely known, is most likely due to hypersensitivity. The allergic reaction could have been caused by exposure to antigens of avian origin or the NTM. This condition is usually under-reported or misdiagnosed due to challenging clinical diagnosis which requires comprehensive array of laboratory diagnostic techniques. Insufficient information on immunologic disorders and other forms of disease conditions in elephants can also lead to misdiagnosis of early

manifestation of hypersensitivity pneumonitis for bacterial or viral pneumonia. Nonetheless, if tentatively diagnosed, early identification and avoidance of the insulting agent is the mainstay of management. Supportive treatment by administration of corticosteroid is recommended in acute and sub-acute forms of hypersensitivity pneumonitis (Weiss and Glassroth, 2012). To date, there is no other elephant in the same captivity experiencing the same condition.

CONCLUSION

Husbandry practices do play a critical role in the control and prevention of diseases in wildlife. Proper care has to be taken to reduce feed contamination with wild bird's excreta and feathers as well as reducing roosting areas in and around the elephant enclosure. There is a need for further studies on how to improve existing captive elephant husbandry practices while maintaining high standards of welfare and providing for the ecological requirements of the species.

ACKNOWLEDGEMENT

We thank all the staff at the zoo and the Department of Wildlife and National Parks, Peninsular Malaysia for the permission to conduct the postmortem and publishing the paper. This study was funded by UPM Research Grant (UPM/700-1/2/RUGS/05-02-12-1833RU).

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