

CASE REPORT

CHROMOBACTERIUM VIOLACEUM INFECTION IN TWO BLACK-HANDED GIBBONS: A VETERINARY CASE REPORT

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ABSTRACT. *Chromobacterium violaceum* is a facultative anaerobic, gram-negative rod-shaped bacteria normally found in soil and stagnant water of tropical and subtropical countries. Infections of *Chromobacterium violaceum* are rare among mammals, but the first human infection were reported in Malaysia in 1927. This clinical case reports two separate cases of *Chromobacterium violaceum* infection in two sub-adult male Black-handed Gibbon (*Hylobates agilis*). Both animals were presented with the history of diarrhea, pyrexia and inappetance. From the physical examination, the findings were high body temperature, dull, inactive, soft fecal stained at the rectum and small open wound at lower limb of one of the case. The treatment given was antipyretic and multivitamin. Unfortunately, due to poor prognosis both patients died within 48 hours after presentation and treatment. Autopsy examinations were performed to find out the cause of death. Post mortem examination findings revealed hepatomegaly with multiple size

whitish-yellow spots on the liver surface, congestion and enlargement of spleen and lymph node, congestion of lung and loss of demarcation between renal cortex and medulla of the kidney. The cellular changes via histopathological findings of kidney, spleen, lung and liver were suggestive of septicaemia. The gross and histopathological findings were supported by the isolation of *Chromobacterium violaceum* via bacterial isolation and identification from lung, liver, spleen and kidney. Thus, the cause of death of the two sub-adult male Black-handed gibbon in this case are due to septicaemia due to *Chromobacterium violaceum* infection.

Keywords: black-handed gibbon, *Chromobacterium violaceum*, septicaemia

INTRODUCTION

Chromobacterium violaceum is a facultative anaerobic gram-negative rod-shaped bacteria commonly found in soil and stagnant water of tropical and subtropical regions. There are two strains of

Chromobacterium violaceum, pigmented and non-pigmented strains. This bacterium is classified as saprophytic bacterium and infections are rare. However, it has the ability to cause an infection in mammals including human (Mario *et al.*, 2010), non-human primate (Groves *et al.*, 1969 and Mario *et al.*, 2010). The first human infection was reported in Malaysia in 1927 (M. Sureisen *et al.*, 2008 and Marco *et al.*, 2011) while the infection in a wild non-human primate was reported in Costa Rica in 2010 (Mario *et al.*, 2010). Black-handed gibbon or also known as agile gibbon (*Hylobates agilis*) is a species of primates from the family of Hylobatidae. Their fur is usually variety in colour, black, brown and reddish brown, with both male and female having white coloured rim around the eyes. The species live at the canopy (Gittins, 1983) of dipterocarp forest (O'Brien *et al.*, 2010) of tropical rainforest of Peninsular Malaysia, Southern Thailand, Kalimantan and Sumatera Indonesia (Geissmann & Nijman, 2008). The exact current population of this species in the wild in Malaysia is unknown as the last estimate range from 6.1-18.9 individual/km² (Chivers, 1974; Gittins and Raemaekers, 1980). Currently, the species is listed as endangered in the IUCN expand red list due to the declining trend of their population as a result of habitat loss and illegal trade (Geissmann and Nijman, 2008). Generally, there are a lack of publications on the occurrence of diseases caused by this bacterial species in the wild. An incidence of *Chromobacterium violaceum* infection in gibbon was first reported by Groves *et al.* (1969) where nine gibbons died of *Chromobacterium violaceum* infection in a zoo in Malaysia after drinking from a

contaminated water source. This veterinary case report describes two separate cases of *Chromobacterium violaceum* infection in two sub-adult male black-handed gibbon.

CASE HISTORY

Two separate clinical cases in black-handed gibbon were reported in 2014 and 2016 with similar clinical signs. Both were sub-adult males (<4 years old), kept in captivity since young and presented with complaints of pyrexia, inappetance and diarrhoea. The first case (2014) had the history of direct contact with soils and stagnant water in a forest reserved one month prior to developing clinical signs. The second case (2016) had no history of direct contact to either bare soils or stagnant water prior to the clinical signs. Physical examination revealed high temperature of more than 40 °C for both cases and in the second case the veterinarian found the patient to have a small open wound (<1 cm in diameter) at the cranial part of of the left knee joint. The animals were treated with anti-pyretic (Flunixin meglumine, s.i.d, 2 mg/kg intramuscularly) and immune-boast supplement (Mamimune, b.i.d, 2 ml orally). Unfortunately, both patients did not respond well to the treatment and died within 48 hours after presentation.

Autopsies were performed on both cases which revealed hepatomegaly with multiple sizes (2-5 mm in diameter) whitish-yellow foci on the liver surface (Figure 1), congestion and enlargement of spleen, lymph node, lungs and loss of demarcation between renal cortex and medulla of the kidney. Examination of gastrointestinal

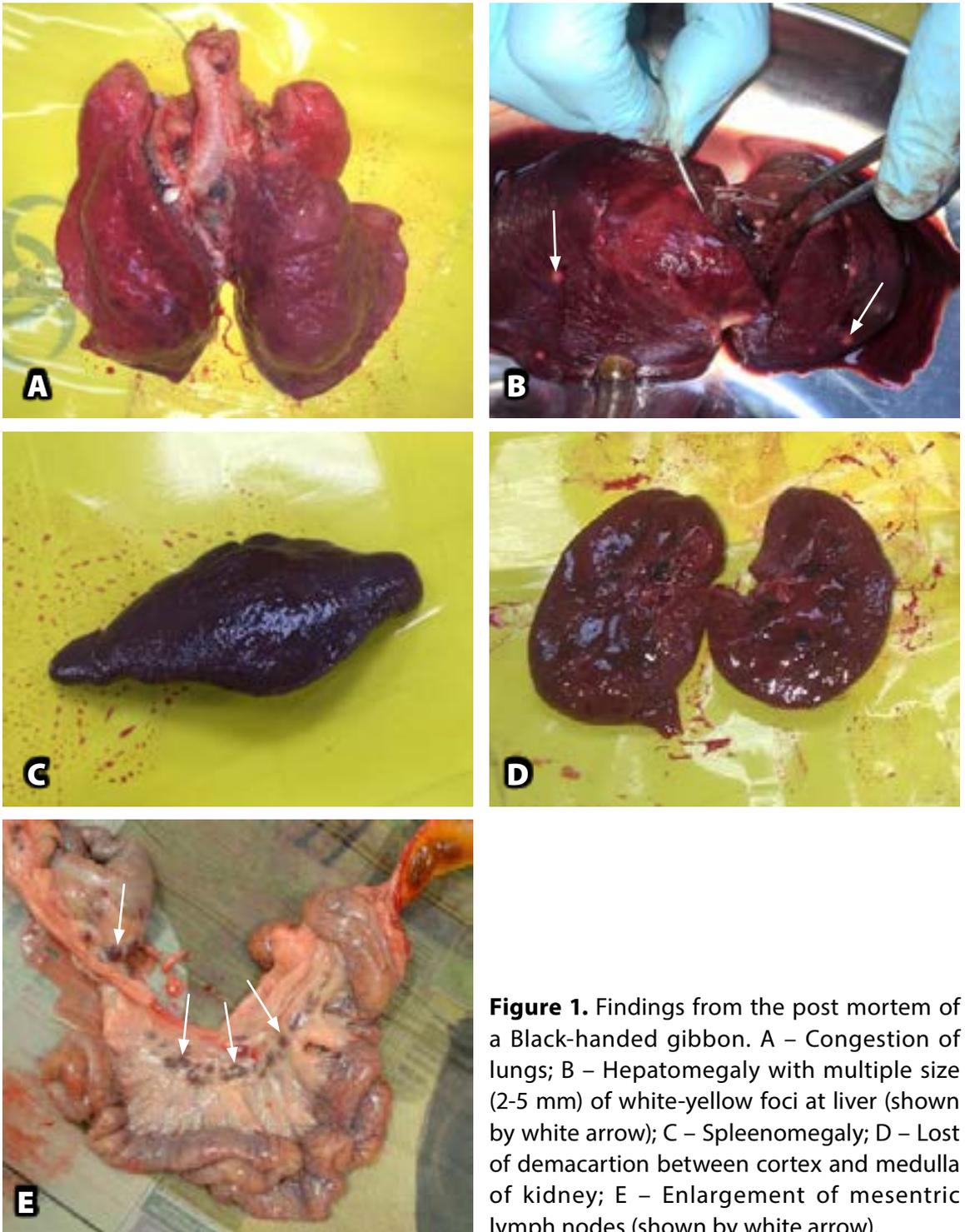


Figure 1. Findings from the post mortem of a Black-handed gibbon. A – Congestion of lungs; B – Hepatomegaly with multiple size (2-5 mm) of white-yellow foci at liver (shown by white arrow); C – Splenomegaly; D – Lost of demarcation between cortex and medulla of kidney; E – Enlargement of mesenteric lymph nodes (shown by white arrow).

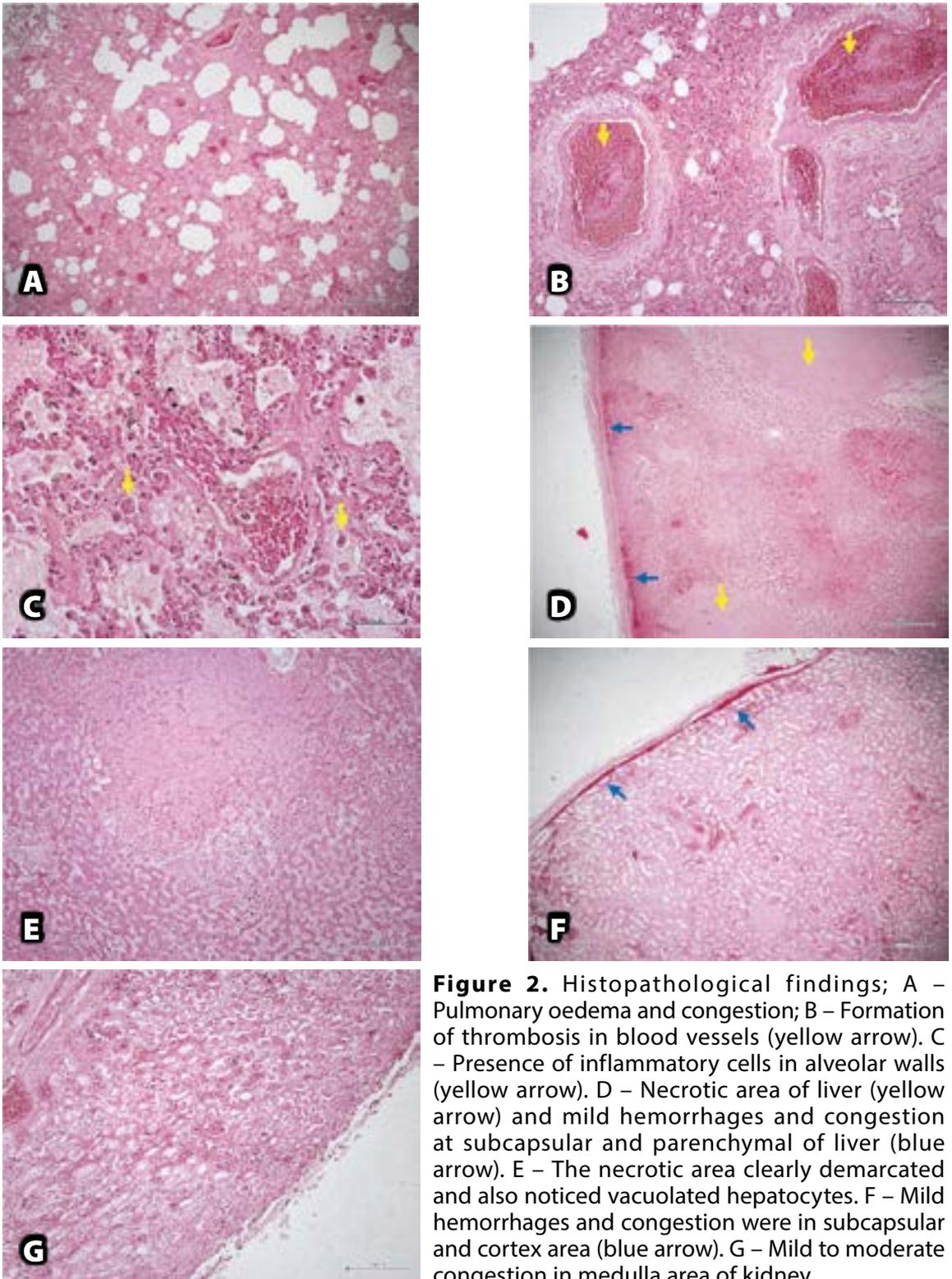


Figure 2. Histopathological findings; A – Pulmonary oedema and congestion; B – Formation of thrombosis in blood vessels (yellow arrow). C – Presence of inflammatory cells in alveolar walls (yellow arrow). D – Necrotic area of liver (yellow arrow) and mild hemorrhages and congestion at subcapsular and parenchymal of liver (blue arrow). E – The necrotic area clearly demarcated and also noticed vacuolated hepatocytes. F – Mild hemorrhages and congestion were in subcapsular and cortex area (blue arrow). G – Mild to moderate congestion in medulla area of kidney.

tract revealed enlargement of mesenteric lymph nodes and pinpoint haemorrhage at mucosal layer of stomach and caecum. Only the second case showed enlargement of heart, haemathorax and haemorrhage of the inner part of the thoracic wall. Lung, liver, spleen, kidney and intestine samples (heart for the second case) were sent for bacteriological isolation and identification and histopathological examination. Bacteriological isolation and identification revealed presence of *Chromobacterium violaceum* from liver, lung, spleen and kidney and heart samples from both cases. Moderate *Pseudomonas* sp. was also isolated from organs of the first case while *E.coli* was isolated from intestinal sample of the second case.

Histopathology findings revealed congestion, oedematous lung with presence of thrombosis and macrophages in alveolar walls (Figure 2). Both kidney and liver showed cellular changes of congestion and mild haemorrhage. Presence of necrotic area were clearly demarcated by vacuolated hepatocytes at the liver. Submucosal congestion of intestine and generalised white and red pulps of spleen was only noticed in the first case.

From the history, physical examination findings, gross pathology, histopathology and bacterial isolation and identification, it was concluded that the cause of death of both sub-adult male black-handed gibbons were due to septicaemia caused by a *Chromobacterium violaceum* infection.

DISCUSSION

Chromobacterium violaceum is a saprobe bacterium. However, it can cause severe infection in mammals by gaining entry through breakage of skin barrier or from ingestion of contaminated water or soil by immunocompromised mammals (Lee *et al.*, 1999; Dyer *et al.*, 2000; De Sequiera *et al.*, 2005 and Mario *et al.*, 2010). The common pathological changes that can be seen in animals dying due to *Chromobacterium violaceum* infection are multiple white foci and necrosis in liver (Groves *et al.*, 1969; McClure and Chang, 1976; Mario *et al.*, 2010; Liu *et al.*, 2012); these lesions are in accordance with the lesions observed in the cases (McClure and Chang, 1976; Dyer *et al.*, 2000 and Liu *et al.*, 2012). In some cases, abscess can be seen in spleen, subcutaneous, tonsil and lymph nodes.

The gibbon in the first case could have been infected through ingestion of contaminated water or soil as no external injury was found during physical examination. While in the second case, the gibbon might have been infected through both routes, orally and an open wound at the lower limb. Since early treatment with antimicrobials was not performed, systemic infection would have taken place causing septicaemia resulting in organ failure and death.

This bacterium has zoonotic potential and can cause fatal infection in immune-compromised individuals. Human cases have been reported in tropical countries with the first case reported in Malaysia in 1927. A study conducted by Yang and Li (2011) on human infection due to *Chromobacterium*

violaceum from 1952 till December 2009 showed 106 patients infected with *Chromobacterium violaceum*, of which 56 of them died due to the infection. The clinical symptoms in human cases include sepsis, lymphadenitis, organs abscess, cellulitis at trauma site, sinusitis, meningitis and urinary tract infection (Julio A. Díaz-Pérez *et al.*, 2007). Human could get infected via direct contact, ingestion of contaminated water or through open wounds.

Diagnosis of this disease is challenging because no pathognomonic lesion or serological tests are available. However, PCR can be used as rapid diagnosis. Failure to diagnose patients infected with *Chromobacterium violaceum* can cause misdiagnosis of other causative agents causing systemic disease such as melioidosis. Good antimicrobial agents against *Chromobacterium violaceum* include fluoroquinolone, carbapenem and ciprofloxacin (Aldridge *et al.*, 1988; Adriana *et al.*, 2006; Yang and Li, 2011).

Chromobacterium violaceum can cause severe systemic infection in the black-handed gibbon leading to multiple organ failure and death. Early and accurate diagnosis and detection of infection are crucial in order to tailor the proper treatment regime and reduce mortality. The bacteria are zoonotic, therefore proper personnel protective equipment must be worn when handling infected animals.

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