

Research Article

Bats (chiropteran) reported with *Aspergillus* species from Kubah National Park, Sarawak, Malaysia

JAYA SEELAN Sathiya Seelan¹, Faisal Ali ANWARALI KHAN², SEPIAH Muid³, M.T. ABDULLAH⁴

¹Institute for Tropical Biology and Conservation, Locked bag 2073, Universiti Malaysia Sabah, 88999 Kota Kinabalu, Sabah, Malaysia

²Department of Biological Sciences, Texas Tech University, Lubbock, Texas 79409-3131, USA

³Department of Plant Science and Environmental Ecology

⁴Department of Zoology, Faculty of Resource Science and Technology, 94300 Kota Samarahan, Sarawak, Universiti Malaysia Sarawak, Malaysia

ABSTRACT

A preliminary survey of chiropterans (bats) with potential zoonotic fungi was conducted as part of the Sowell-UNIMAS Expedition 2006. This survey was conducted at Kubah National Park, Matang, Sarawak from 14th to 16th August 2006. The main aim of this survey was to document variety of fungal isolates from bats external (ears) and internal (saliva and anal) swabs. All of the fungi species were subjected to both macroscopic and microscopic observations to characterize their morphology. Out of 23 species of bats observed, 13 (56.5%) species were found to contain 17 fungi isolates of the genus *Aspergillus* from five subgenera, five sections and six species. The fungi isolates were *Aspergillus restrictus*, *A. sydowii*, *A. fumigatus*, *A. niger*, *A. clavatus* and *A. japonicus*. The highest numbers of isolates recorded was for *A. restrictus* with six isolates followed by *A. fumigatus* and *A. sydowii* with two isolates respectively. Where as, *A. niger*, *A. clavatus* and *A. japonicus* each recorded with one isolate only. *Aspergillus fumigatus* was the

first record isolated from bats the samples (n = 64) from Sarawak. It was reported that this isolate is a pathogenic and thermophilic (able to grow up to 65°C) isolate which was found to be on a lesion near ear opening of *Hipposideros cervinus*. Further work should be done to discover potential mycoflora in wildlife mammals.

INTRODUCTION

Wildlife has been an important vector of infectious diseases as they can transmit these diseases to human through direct or indirect contact. Today, zoonoses found in wildlife constitute a major public health problem, affecting all continents. Wild animals seem to be involved in the epidemiology of most zoonoses and serve as major reservoirs for transmission of zoonotic agents to domestic animals and humans (Frederick, 1998; Hilde *et al.*, 2004).

The importance of such zoonoses is increasingly being recognized, and the needs for more attention in this area have been widely expressed (Hilde *et al.*, 2004; Chomel *et al.*,

Keywords: *Aspergillus*, bats, pathogenic, thermophilic

2007). Recent studies showed that wildlife diseases were expanding in geographic range, transferred from one host species to another, increased in impact or severity, undergone a change in pathogenesis, and might also have emerged by recently evolved pathogens (Lederberg *et al.*, 1992; Krause, 1992; Krause, 1994; Daszak *et al.*, 2000). Most of the outbreak of new viruses and emergence of newly evolved pathogens are known mainly from wildlife population (Morse, 1993; Dobson & Foufopoulos, 2001; Antia *et al.*, 2003).

Fungi are distributed worldwide, with particular species being endemic in particular regions. The species are grouped by natural environment habitat as being primarily associated with humans (anthrophilic), other animals (zoophilic), or soil (geophilic) (Brandt & Warnock, 2003). Zoosporic fungi are one of the key areas around these issues that are currently receiving more attention with regards to the wildlife diseases and yet to be explored as other charismatic pathogens (e.g. virus, bacteria and protozoan). Worldwide distribution of some common wild animal fungal diseases such as *Aspergillosis*, *Chytridiomycosis*, *Coccidioimycosis*, *Cephalosporiosis* and *Otomycosis* have the potential to be a major threat to human being as they starts to be transmitted from animal to human (Gitter & Austwick, 1962). Previous cases of fungal infections in wild animals have caused inflammatory lesions in the brains, subcutaneous infections, urinary tract infections, otitis, infraocular infections and genital infections (Halloran, 1955; Conen *et al.*, 1962). For example, chytridiomycosis has caused near extinction of some amphibians (*Dendrobates tinctorius*) in Australia and other parts of the world (Ells *et al.*, 2003).

Fungal species in wild animals (from samples of tissues, saliva and anal swab) such as *A. fumigatus*, *A. terreus*, *A. restrictus*, *A. versicolour* and *A. glaucus* were the common fungi that have been isolated from temperate

region (Raper & Fennell, 1977). *Aspergillus fumigatus* is the most important group of fungi as a cause of systemic human and animal diseases (Raper & Fennell, 1965; 1977). Since *Aspergillus* species can be found on many different substrates, they have been extensively studied from animal dung, lungs, ears, intestinal, blood vessels, kidney, bladder, brain and skin for pathogenic strains that leads to fungal infections on wild animals (Ainsworth, 1952). Among wildlife population, several studies have been documented different pathogenic fungi from different hosts (Coon & Locke, 1968; Trevino, 1972; Kaliner & Cooper, 1973; Tham & Purcell, 1974; Ramirez *et al.*, 1976; Peden & Richard, 1985; Speare *et al.*, 1994; Mancianti *et al.*, 1997; Cork *et al.*, 1999; Monteros *et al.*, 1999; Leotta *et al.*, 2002).

Bats have been shown to be both important residence and vectors of pathogens. Some of the pathogens that have been recently reported to be associated with this fauna were rabies (Paez *et al.*, 2003), European lyssavirus (Fooks *et al.*, 2002), Hendra (Halpin *et al.*, 2000) and Menangle (Bowden *et al.*, 2001) in Australia, Nipah and Tioman viruses in Malaysia (Chua *et al.*, 2002a and b), hantaviruses in Korea (Kim *et al.*, 1994) and etc. (Chua *et al.*, 2005). With respect to fungal pathogens, insectivorous bats are known to be the prime contenders as reservoirs of fungi such as *Histoplasma capsulatum*, *Coccidioides immitis*, *Cryptococcus laurentii* and *Blastomyces dermatitidis* (Yamamoto *et al.*, 1995; Garcia-Hermoso *et al.*, 1997; Mattsson *et al.*, 1999; Bunnell *et al.*, 2000). Besides from becoming contagious to human, these diseases also have resulted in mass mortalities, population declines, and even extinctions in such wildlife population (Wobeser, 1994).

Viewing such a condition is important for both human and wildlife, the present study has been initiated in order to screen for pathogenic strains of *Aspergillus* residing in bats of Borneo Island. This genus is known to be commonly

distributed in the environment as well as pathogenic (Raper & Fennell, 1965; Klich, 2001).

MATERIALS AND METHODS

Study Area

Bats were sampled in Kubah National Park, Sarawak, Malaysian Borneo (01°36'42.3"N 110°26'39.3"E—elevation 8-35 m) from 14th to 16th August 2006. The study area was mainly covered with mixed dipterocarp forest with a small stream flowing through this park from Mt. Serapi.

Sampling Method

Ten mist-nets and four harp traps were set in each night. Nets and traps were deployed at night from 6.00 p.m. and were closed at 7.00 a.m. the following day. Nets were checked every 20 minutes during the first 6 hours. Harp traps are much more independent, rarely any bat caught in the pouch can escape and they are not tangled (nor stressed), so these traps were checked at least three times each night, so as to make sure the animals were fine. Bats were also sampled using scoop nets inside roosts (like culverts).

All the samples were identified to species in the field following the identification keys from Payne *et al.* (1998); Corbet & Hill, (1992); Kingston *et al.* (2006); and Khan (1986). A maximum of five individuals per species were taken as voucher specimens. An extra of another three individual were collected for the species, which is identified to have different morphological attributes within the population. Samples were then tagged and field standard measurements were recorded. All specimens were prepared as museum voucher specimens, either as skin and skeletal or as fluid preserved specimen. Tissue samples from liver and muscle were preserved in lysis buffer (Longmire *et al.*, 1997) and ethanol vials, blood samples were collected using Nobuto blood filter strips and

ectoparasites from some specimens were preserved in 75% ethanol.

Anal, ear and saliva swabs were performed using sterile cotton buds and inoculated into screw-capped centrifuge tubes containing 900 µl of PBS buffer to prevent drying. Samples were serially diluted 10-fold up to 10⁻⁵. Of the 10⁻³ to 10⁻⁵ dilution, 0.1 ml of aliquots were spread on sabouroud's agar plates. Three replicates were prepared for each dilution and incubated at 25°C and 37°C for five days. After five days, the mycelia were transferred into selective media for fungal identification.

Fungi Identification

The fungi isolates were grown on five different media i.e., Czapek Yeast Extract Agar incubated at 25°C (CYA25), CYA37 incubated at 37°C, CY20S added with 20% sucrose incubated at 25°C, Czapek's agar (CZ) and Malt Extract Agar (MEA). For each culture, five plates were used as replicates were made. Each plate was inoculated at three points, equidistant from the centre and incubated in the dark for seven days. The strains were identified using current universal identification keys described by Raper & Fennell (1965) and Klich (2002).

Microscopic Observation

Microslide culture technique was used to observe the micromorphological features of the *Aspergillus* species. A small tuft of mycelium and conidiophores were lifted from a young section of the colony, placed in a drop of alcohol on a microscope slide and gently teased out. A drop of lactophenol blue or acid fuchsin was used as a stain. The appearance of foot cell, conidiophores, presence of metulae (sterigmata) and conidia were examined and measurements were recorded. Images were taken using digital camera.

RESULTS

Thirteen species from a total of 23 species of bats caught were found to host 17 *Aspergillus* isolates. Isolates were identified to five subgenera, five sections and six species. The isolates were identified as *A. restrictus*, *A. sydowii*, *A. fumigatus*, *A. niger*, *A. clavatus* and *A. japonicus* (Figures 1–6). *Aspergillus restrictus* was recorded with the highest number of isolates (six isolates), followed by *A. fumigatus* (two isolates) and *A. sydowii* (two

isolates) and *A. niger*, *A. japonicus* and *A. clavatus* were recorded with lowest number of isolates (one each) (Table 1).

Fungal infection was observed near the ear opening of a single insectivorous bat, *Hipposideros cervinus*. This fungus was incubated at 37°C and was confirmed that this infection was due to the *A. fumigatus*. Morphological characteristics of all the six species isolated from the bats are described.

Table 1: Total number of *Aspergillus* species isolated from anal, ear and saliva of the bats

Family Host Species Name Pteropodidae	Fungal Species	Source	No. of Isolates
<i>Cynopterus brachyotis</i>	<i>A. restrictus</i>	Anal	1
<i>C. brachyotis</i>	<i>A. restrictus</i>	Anal	1
<i>C. brachyotis</i>	<i>A. restrictus</i>	Ears	1
<i>C. brachyotis</i>	<i>A. restrictus</i>	Ears	1
<i>C. brachyotis</i>	–		0
<i>C. brachyotis</i>	–		0
<i>C. brachyotis</i>	<i>A. sydowii</i>	Ears	1
<i>Penthetor lucasi</i>	<i>A. restrictus</i>	Anal	1
<i>P. lucasi</i>	<i>A. clavatus</i>	Anal	1
<i>P. lucasi</i>	<i>A. fumigatus</i>	Ears	1
<i>Balionycteris maculata</i>	<i>A. sydowii</i>	Ears, anal	2
<i>Hipposideridae</i>			
<i>Hipposideros cervinus</i>	–		0
<i>H. cervinus</i>	–		0
<i>H. cervinus</i>	<i>A. restrictus</i>	Anal	1
<i>H. cervinus</i>	–		0
<i>H. cervinus</i> **	<i>A. fumigatus</i>	Ears, anal, saliva	4
<i>H. cervinus</i>	–		0
<i>H. cervinus</i>	–		0
<i>Rhinophidae</i>			
<i>Rhinolopus sedulous</i>	<i>A. japonicus</i>	Anal	1
<i>R. trifolius</i>	–		0
<i>Rhinolopus borneensis</i>	–		0
<i>Vespertilionidae</i>			
<i>Myotis muricola</i>	<i>A. niger</i>	Ears	1
<i>Glischropus tylopus</i>	–		0

**Detected with fungal infection; – is negative

Aspergillus japonicus Saito**Subgenus:** Circumdati **Section:** Nigri

Colony on Czapek's yeast extract agar (CYA) 60–75 mm in diameter (7 days, 25°C), conidial heads on this media was very dark brown to purple brown or purple black; mycelium white to yellowish; no sclerotia was observed; reverse dull brown to purple brown; no exudates present; colonies velutinous to slightly floccose, plane to radially sulcate.

On CYA37, the colony diameter was 40–50 mm in diameter (7 days, 37°C); reverse brown to black, yellow to brown soluble pigment present; slow growth colony and radially sulcate. On CY20S, colony was rapidly growing and no soluble pigment was observed. On MEA, this isolate can grow about 70–80 mm in diameter (7 days, 25°C); conidial heads was brownish black; mycelium yellow in colour. No growth at 5°C and this isolate can grow moderately at 45°C.

Conidial heads were radiate; stipes 400–600 µm, smooth-walled, uncoloured. Vesicles 15–25 µm wide, globose shaped, uniseriate; Phialides (4.5–7) × (3–4) µm, covering the upper half of the vesicle. Conidia were globose to subglobose, occasionally ellipsoidal, measuring (3–4) × (4–5) µm, surface echinulate. This isolate produces black spinose conidia (Figure 1, pg. 88).

Aspergillus niger Tiegh**Subgenus:** Circumdati **Section:** Nigri

Colony on Czapek's yeast extract agar (CYA) 57–58 mm in diameter (7 days, 25°C), wrinkled, dense and velutinous, exudates present, white at first and becomes dark brown with forming of conidial heads, reverse dark yellow.

Colony on malt extract agar (MEA) 52–56 mm in diameter (7 days, 25°C) similar to those on CYA but less dense and conidia in duller colours, reverse dirty yellow. On Czapek's, morphological characters were similar to those on MEA.

No growth at 5°C. Growth at 37°C is exceptionally rapid, colonies on CYA 38–40 mm in diameter in three days. This strain can grow at 45°C.

Conidial apparatus develops as erect conidiophores. Tips of conidiophores enlarge and form vesicles with many phialides producing conidia in long chains. Conidial heads are compactly columnar, 40–48 µm in diameter, black. Conidiophores are unbranched, smooth and dark brown, stipes 200–300 µm × 3–4 µm in size. Vesicles are round to globose shaped, 20–30 µm in diameter. Phialides crowded dark brown, 5–7 µm long. Conidia are globose to subglobose, roughened, hyaline and often deciduous spinules when young and verruculose when it matures (Figure 2, pg. 88).

Aspergillus sydowii (Bain. & Sart.) Thom and Church**Subgenus:** Nidulantes **Section:** Versicolores

Colony on Czapek's yeast extract agar (CYA) 20–40 mm in diameter (7 days, 25°C), heavily sporulating in dark turquoise to dark green colours, mycelium white, forming white conidial heads, reverse reddish brown to maroon in colour; exudates reddish brown to dark brown when present; soluble pigment was maroon on CYA; colony dense, velutinous to lanose, radially sulcate.

Colony on malt extracts agar (MEA) 30–40 mm in diameter (7 days, 25°C); conidia on MEA was dark greenish after seven days; mycelium white; colony texture granular and plane. Colony on CY20S was similar to colonies on CYA except exudates usually not formed and texture was more floccose. Colony on CY37 was less dense and the conidial colours less intense. Colony on Czapek's agar growing 30–40 mm with close textured and velvety with crowded conidiophores. No growth at 5°C. Growth at 37°C is moderate. This strain cannot grow at 45°C.

Conidial apparatus develops as erect conidiophores. Conidial heads are radiate, 100

– 150 μm in diameter, and white in colour. Stipes (200–30) $\mu\text{m} \times (3-7) \mu\text{m}$ smooth, thick-walled colourless, expanding into spatulate vesicles. Vesicles are hyaline, radiate to spatulate shaped, 6–15 μm in width; usually sterigmata in two series (biseriate), metulae (4–5) $\times (2-3) \mu\text{m}$ in diameter, phialides (5–7) $\times (2-3) \mu\text{m}$. Diminutive conidial structures produced by this isolate, looks like penicillate heads. Conidia are spherical, very rough to spinose, 3–4 μm in diameter. Hulle cells present in this isolate (Figure 3, pg. 88).

Aspergillus restrictus G. Smith

Subgenus: *Aspergillus* **Section:** *Restricti*

Colony on Czapek's yeast extract agar (CYA) 4–8 mm in diameter (7 days, 25°C), Conidia on CYA25 dull green to grey green; mycelium white or inconspicuous; reverse uncoloured to brown; exudates absent; colonies velutinous, centrally floccose.

Colony on malt extract agar (MEA) 3–6 mm in diameter (7 days, 25°C); conidia on MEA was dark greenish after seven days; mycelium white; reverse uncoloured to pale tan colour; irregular margin. Conidia on CY20S, dark green, mycelium inconspicuous, reverse uncoloured, colony very slow growth, dense and plane. Colony on Czapek's agar growing 3–5 mm; conidia dark green; mycelium inconspicuous, slow growth and less sporulation. No growth at 5°C. Growth at 37°C is very slow. This strain cannot grow at 45°C.

Conidial heads are columnar; conidiophores up to (100–200) $\times (4-7) \mu\text{m}$, smooth and colourless, stipes (200–30) $\mu\text{m} \times (3-7) \mu\text{m}$ smooth, uncoloured, expanding into pyriform or hemispherical vesicles. Vesicles 8–18 μm in wide; uniseriate to biseriate. Phialides (6–10) $\times (2-3) \mu\text{m}$ in diameter. Conidia are variable, often cylindrical when borne, at maturity ellipsoidal or pyriform, rough walled, (4–6) $\times (2-3) \mu\text{m}$. (Figure 4, pg. 88).

Aspergillus fumigatus Fresenius

Subgenus: *Fumigati* **Section:** *Fumigati*

Colony diameter on CYA attaining about 50–75 mm (7 days at 25°C); conidia on this media was dark blue greenish; mycelium white; exudates absent; reverse uncoloured; soluble pigment absent; texture floccose; heavy sporulation.

On MEA, colony growth was about 50–60 mm (7 days at 25°C); conidial colour was dark bluish green; reverse uncoloured; texture as on CYA25. On CY20S and Cz, the colony colour was similar as in CYA25. Colony on CYA37 was exceptionally rapid, heavy sporulation; conidia colour was greyish green. This isolate cannot grow at 5°C and able to grow until 65°C.

Conidial heads was columnar; conidiophores uncoloured, smooth-walled (200–350) $\times (6-10) \mu\text{m}$, pyriform to spatulate vesicles. Vesicles 15–28 μm in diameter; uniseriate; phialides (5–7) $\times (2-3) \mu\text{m}$, all phialides are parallel to each other and the conidiophore axis. Conidia globose to broadly ellipsoidal, smooth to finely roughened, 2–2.5 μm in diameter (Figure 5, pg. 89).

Aspergillus clavatus Desm

Subgenus: *Clavati* **Section:** *Clavati*

Colony on CYA (7 days at 25°C) was 40–45 mm; conidia dull green, dark turquoise or green; mycelium white, inconspicuous to floccose; exudates absent; reverse dull yellow to orange brown in colour; colonies dense, radially furrowed. Yellow soluble pigment present.

On MEA, colony attaining about 40–50 mm in diameter (7 days at 25°C); conidia were dull green to grey green in colour, irregularly distributed; dark yellow soluble pigment present; mycelium white, inconspicuous; colony thin and low except for conidial areas more floccose. On CY20S and CZ colonies were similar as those on CYA25. Colony on CYA (7 days at 37°C) was

very slow, conidia less abundant. This isolate cannot grow at 5°C and colony growth was moderate at 37°C.

Conidial heads radiate, splitting into columns when matured; conidiophores rose up to $(1000 - 2000) \times (15 - 30) \mu\text{m}$, smooth-walled, colourless, expanding slowly into clavate

vesicles. Vesicles $50 - 70 \mu\text{m}$ wide, smaller vesicles, conidia zone extended from $30 - 175 \mu\text{m}$ down from the apices of the vesicles; uniseriate, phialides $(8 - 11) \times (2 - 3) \mu\text{m}$. Conidia smooth-walled, ellipsoidal, occasionally pyriform, almost cylindrical, $(3 - 5) \times (3 - 3.5) \mu\text{m}$ (Figure 6, pg. 89).

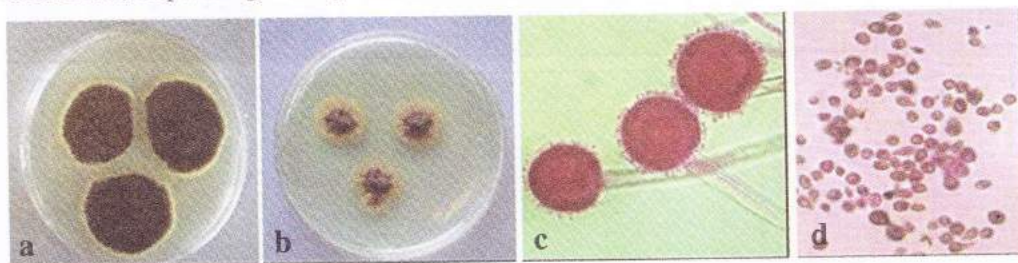


Figure 1: (a – b) Colonies on CYA at 25°C; 37°C; (c) Biseriate conidial head; (d) Conidia

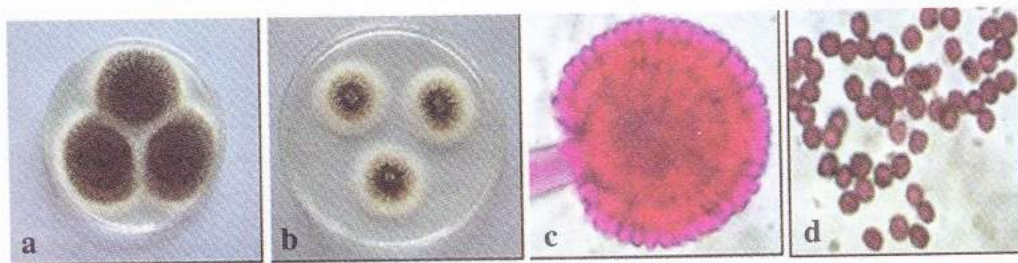


Figure 2: (a – b) Colony on CYA at 25°C; 37°C; (c) Biseriate conidial head; (d) Conidia

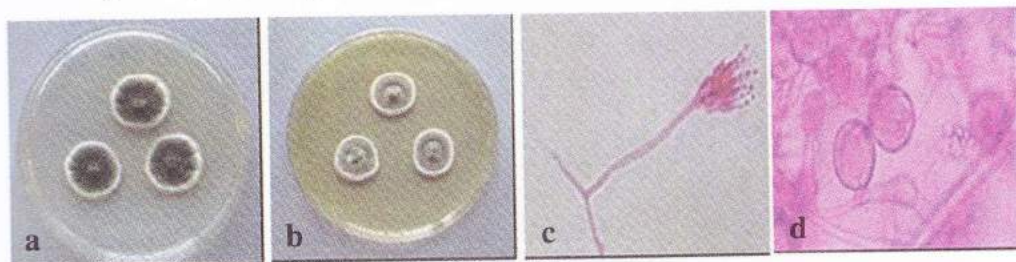


Figure 3: (a – b) Colony on CYA at 25°C; colony on MEA at 37°C; (c) Conidial head; (d) Hulle cells

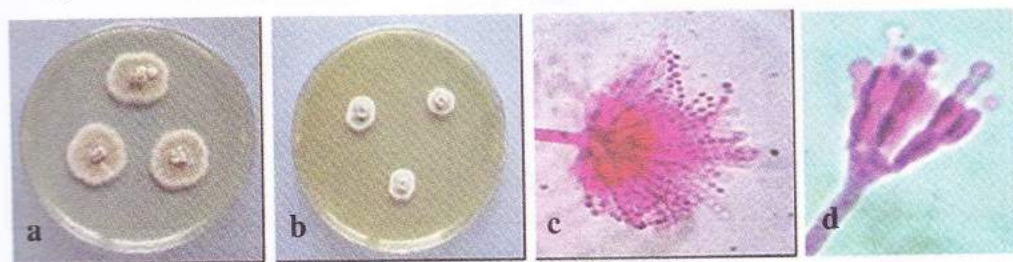


Figure 4: (a) Colony on CYA at 25°C; (b) Colony on MEA at 25°C; (c) Conidial head; (d) Biseriate conidial head

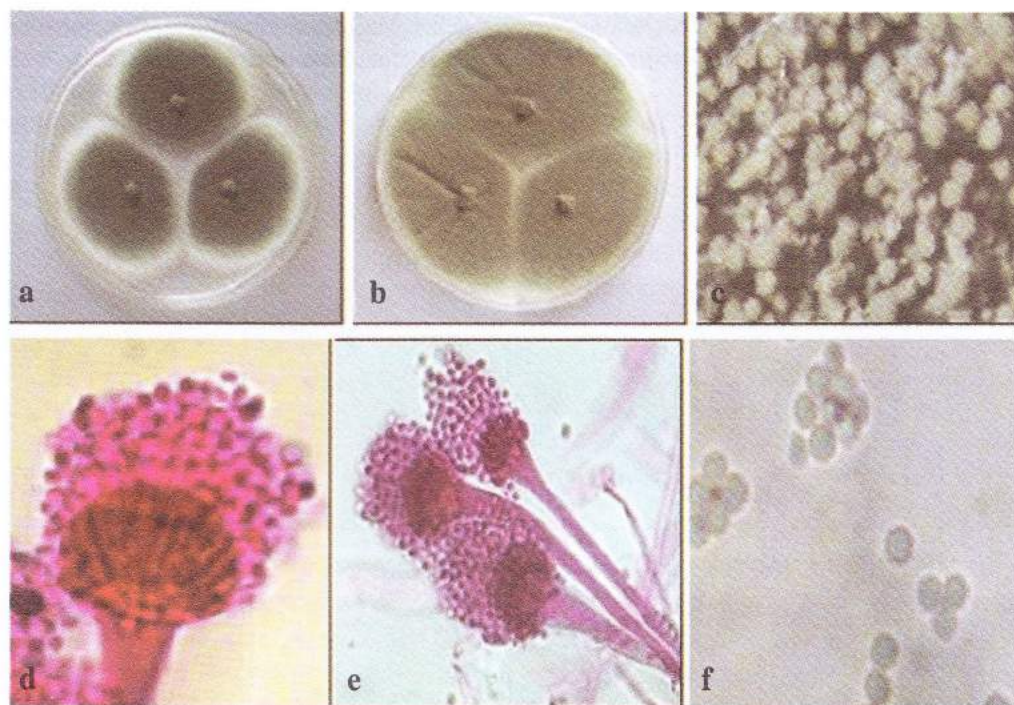


Figure 5: (a – b) Colony on CYA at 25°C; 37°C; (c) Conidial heads columnar; (d – e) Uniseriate conidial head; (f) Conidia shape globose

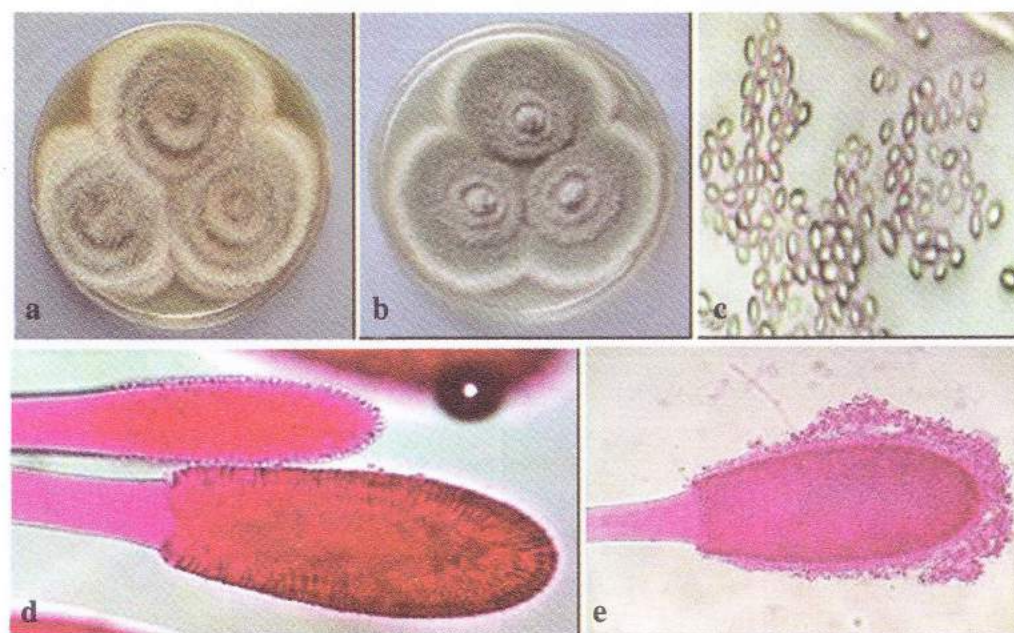


Figure 6: (a) Colony on MEA at 25°C; (b) Colony on CYA at 25°C; (c) Conidia shape ellipsoidal; (d – e) Uniseriate conidial head

DISCUSSIONS

In this preliminary study, six species of *Aspergillus* were isolated from 13 species of bats. As few studies have been conducted on mycoflora from wild animals, all the *Aspergillus* species recorded in this study were presumed to be first record for this genus found in bats at least in Sarawak, Borneo. Previous studies done by Kuthubutheen & Webster (1986) have isolated coprophilous fungi from the bats, sambar deer and elephants dung in the natural environment. Furthermore, *Candida lusitanae* and *Debaryomyces hansenii* were isolated from bat guano (Takashi *et al.*, 2005). Several pathogenic fungi from different bats species were reported from Brazilian Amazon. In the search of Paracoccidioides from the bats' viscera, Silva-Vergara *et al.* (2005) have also documented other types of mycoflora such as *Candida krusei*, *Trichosporum* sp., *Scytalidium* sp., and one unidentified yeast-like fungus from liver, spleen and lungs of frugivorous bats (*Carollia perspicillata* and *Sturnira lilium*), *Glossophaga soricina* (nectarivorous bats) and *Desmodus rotundus* (hematophagous bat) respectively.

In another study by Koilraj *et al.* (1999) on the distribution of *Aspergillus* in caves soil from India, documented 13 different species of *Aspergillus* such as *A. niger*, *A. japonicus*, *A. flavus*, *A. versicolour*, *A. tamarii*, *A. sydowii*, *A. chevalieri*, *A. ochraceous*, *A. parasiticus*, *A. fumigatus*, *A. terreus*, *A. wentii* and *Aspergillus* sp. Besides that, a study done by Moraes *et al.* (2001), have proved that arthropod vectors like *Culex* and *Aedes* have become as carrier for the *Aspergillus* species like *A. niger*, *A. flavus*, *A. nidulans*, *A. fischerianus* and *A. heteromorphus*. Hence, the transmission of the fungal spores in bats may be due to the mosquitoes bites in their habitat where they live in.

Although our sample size is not really large to conclude statistically in which swab location

(ear, saliva or anal) contains the highest number of *Aspergillus* species, but this preliminary data showed that the most diversity of this fungus were observed in anal swabs of frugivorous bats. Thus, this indicates that various types of substrates such as anal, ears, saliva and faeces (guano) results different types of mycoflora in the wild animal population. The high number of fungal isolates documented in frugivorous bats compared to insectivorous bats also does show some correlation on food sources and their roosting site with the number of fungus isolated in this study.

Based on the morphological examinations, some colony and microscopic characteristics of the *Aspergillus* species were found to be different from those stated by Raper & Fennell (1965) and Klich (2002). For example, the colony diameter of *A. restrictus* on CYA was smaller. It was determined that the colony diameter of *A. restrictus* ranged between 4.0 – 8.0 mm on CYA and the diameter of the vesicles varied up to 8 – 18 µm. The most distinguishing property of *A. clavatus*, dull green or grey green conidial heads on MEA as stated by Raper & Fennell (1965) was observed in this strain. At the same time, a strong yellow soluble pigment present on this media was another important new characteristic that was observed in this study probably might be a different strain.

Moreover, there was a similar morphological characteristics were observed in *A. japonicus*, *A. niger*, *A. sydowii* and *A. fumigatus* as described by Raper & Fennell (1965), Samson & Pitt (1990), Klich (2002). However, a pathogenic strain of *A. fumigatus* that was isolated from the *Hipposideros cervinus* showed thermophilic properties as these isolates able to survive under a wide range of temperature (25 – 65°C). According to Gilman (1959), *A. fumigatus* strains can grow rapidly up to 35 – 60°C. This study showed that the *A. fumigatus* isolated from *Hipposideros cervinus* could grow up to 65°C.

In Borneo, the most widespread species of the genus *Aspergillus* are *A. niger* Tieghem, *A. flavus* Link, *A. fumigatus* Fres, *A. ochraceus* Wilhelm, *A. terreus* Thom and *A. wentii* Wehmer (personal communication). These isolates have been isolated from different types of soil, plants (fresh and senescent leaves), fruits, leaf litters, water samples, food products, indoor and outdoor environments. Thus, *Aspergillus* could also be isolated from wild animal population because it is thought that these species are more adapted to the prevailing ecological conditions (Sepiah, 1985; Jaya Seelan & Sepiah, 2006).

This study provides a comparative analysis on *Aspergillus* species available in the chiropterans samples from Kubah National Park might be due to climatic agents such as flood and air or wind which can help to introduce fungal spores and mycelium into the cave environment. Besides that, fungal spores might also enter into cave through organic substances such as plant which are carried into the cave by troglodytes (Cubbon, 1970).

Since bats are widely distributed in the cave environment, fungal spores might cause infection for bats when they are exposed or even live as symbionts. Yamamoto *et al.* (1995) investigated that the bat guano may mediate the exchange of pathogenic fungi just as pigeon excreta mediate the exchange of *Cryptococcus neoformans*, the causal agent of cryptococcosis.

Apart from that, fruits consumed by the frugivore bats are also important factor in understanding the ecology of bats. Sometimes the infected fruit may contain pathogenic microorganisms that present during the fruit decay process (Sepiah, 1985). So, this would be a key factor how the fungi are transmitted to bats since frugivorous bats consume fruits as their main diet.

CONCLUSION

This study has provided a preliminary list of *Aspergillus* species found on chiropterans. There were six species of *Aspergillus* successfully isolated from the 13 species of bats. This study has documented the diversity of *Aspergillus* species in bats while understanding the macro and microscopic characteristic of this fungus cultured in different media sources. Pathogenic strains of microorganisms could be studied in order to understand their host relationship and also ecological significance in the wild animal population in Malaysia. In general, this preliminary analysis provides novel and basic information on potential sources of mycoflora from the wild animals in Borneo. Malaysian bats may contain several novel fungi species and may be of significant mycological interest. Further study is needed in order to conserve wild animals from emerging infectious diseases.

ACKNOWLEDGEMENTS

Authors would like to thank Universiti Malaysia Sarawak (UNIMAS) and Texas Tech University (TTU) for giving the opportunity to participate in Sowell-UNIMAS Expedition 2006. The authors are also grateful to Prof. Maren Klich of the United States Agricultural Department (USDA) and Prof. Jens Frisvad Denmark Technical University (DTU) for providing information on the *Aspergillus* species identification. Authors would also like to thank all members of all individuals participated in Sowell-UNIMAS 2006 Expedition from TTU, Faculty of Resource Science and Technology, Sarawak Forest Department and Sarawak Forest Cooperation.

REFERENCES

- Ainsworth, G. C. 1952. Medical Mycology: An Introduction to its Problems. London: Pitman. pp 105.

- Alexopoulos, C. J. and C. W. Mims. 1979. *Introductory Mycology*. (3rd Ed.). Canada. pp 625.
- Amengual, B., J. E. Whitby, A. King, J. S. Cobo and H. Bourhy. 1997. Evolution of European bat lyssaviruses. *Journal of General Virology*. 78: 2319 – 2328.
- Antia, R., R. R. Regoes, J. C. Koella and C. T. Bergstrom. 2003. The role of evolution in the emergence of infectious diseases. *Nature*. 426: 658 – 661.
- Bowden, T. R., M. Westenberg, L. F. Wang, B. T. Eaton and D. B. Boyle. 2001. Molecular characterization of Menangle virus, a novel paramyxovirus which infects pigs, fruit bats and humans. *Virology*. 283: 358 – 373.
- Brandt, M. E. and D. W. Warnock. 2003. Epidemiology, clinical manifestations and therapy caused by dematiaceous fungi. *Journal of Chemotherapy*. 15: 35 – 47.
- Bunnell, J. E., C. L. Hice, D. M. Watts, V. Montrueil, R. B. Tesh and J. M. Vinetz. 2000. Detection of pathogenic *Leptospira* spp. infections among mammals captured in Peruvian Amazon basin region. *American Journal of Tropical Medicine and Hygiene*. 63: 255 – 258.
- Chomel, B. B., A. Belotto and F. X. Meslin. 2007. Wildlife, exotic pets and emerging zoonoses. *Emerging Infectious Diseases*. 13(1): 6 – 11.
- Chong, H. T., S. R. Kunjapan, T. Thayarapan, J. M. G. Tong, V. Petharunam, M. R. Jusoh and C. T. Tan. 2000. Nipah encephalitis outbreak in Malaysia, clinical features in patients from Seremban. *Neurological Journal of Southeast Asia*. 5: 61 – 67.
- Chua, P. K. B., J. E. Corkill, P. S. Hooi, S. C. Cheng, C. Winstanley and C. A. Hart. 2005. Isolation of *Waddlia malaysiensis*, a novel intracellular bacterium, from fruit bat (*Eonycteris spelaea*). *Emerging Infectious Diseases*. 11: 2.
- Chua, K. B., C. L. Koh, P. S. Hooi, K. F. Wee, J. H. Khong and B. H. Chu. 2002a. Isolation of Nipah virus from Malaysian Island flying foxes. *Microbes and Infection*. 4: 145 – 151.
- Chua, K. B., L. F. Wang, S. K. Lam and B. T. Eaton. 2002b. Full length genome sequence of Tioman virus, a novel paramyxovirus in the genus *Rubulavirus* isolated from fruit bats in Malaysia. *Archives of Virology*. 147: 1323 – 1348.
- Chua, K. B., K. J. Goh, K. T. Wong, A. Kamarulzaman, P. S. K. Tan, T. G. Ksiazek, S. R. Zaki, G. Paul, S. K. Lam and C. T. Tan. 1999. Fatal encephalitis due to the Nipah virus among pig-farmers in Malaysia. *The Lancet*. 354: 1257 – 1259.
- Conen, P. E., G. R. Walker, J. A. Turner and P. Field. 1962. Invasive primary aspergillosis of the lung with cerebral metastasis and complete recovery. *Diseases of Chest*. 42: 88 – 94.
- Coon, N. C and L. N. Locke. 1968. Aspergillosis in a bald eagle, *Haliaeetus leucocephalus*. *Bulletin of Wildlife Disease Association*. 4: 51.
- Corbet, G. B. and J. E. Hill. 1992. *The mammals of the Indomalayan region: A systematic review*. Oxford: Oxford University Press.
- Cork, S. C., M. R. Alley, A. C. Johnstone and P. H. G. Stockdale. 1999. Aspergillosis and other causes of mortality in the stitch bird in New Zealand. *Journal of Wildlife Diseases*. 35(3): 481 – 486.
- Cubbon, B. D. 1970. *The Science of Speleology*. London: Academic Press. pp 423.
- Daszak, P., A. A. Cunningham and A. D. Hyatt. 2000. Emerging infectious diseases of wildlife: Threats to biodiversity and human health. *Science*. 287: 443 – 449.
- Dobson, A. and Foufopoulus. 2001. Emerging infectious pathogens of wildlife. *Philosophical Transactions of the Royal Society of London*. 356: 1001 – 1012.
- Ells, T. V., J. Stanton, A. Strieby, P. Daszak, A. D. Hyatt and C. Brown. 2003. Use of immunohistochemistry to diagnose chytridiomycosis in dyeing poison dart frogs *Dendrobates tinctorius*. *Journal of Wildlife Diseases*. 30(3): 742 – 745.
- Erkens, K., M. Lademann, K. Tintelnnot, M. Lafrenz, U. Kaben and E. C. Reisinger. 2002. Histoplasmosis group disease in bat researchers returning from Cuba. *Deutsche Medizinische Wochenschrift*. 127: 21 – 25.

- Fooks, A. R., C. Finnegan, N. Johnson, K. Mansfield, L. McElhinney and P. Manser. 2002. Human case of EL type 2 following exposure to bats in Angus, Scotland. *Veterinary Medicine*. 151: 679.
- Forgacs, J. 1962. *Mycotoxinoses in animal and human health*. Proceedings of United States Live Stock Sanitary Association, 66th Annual Meeting. pp 426 – 448.
- Forgacs, J. and W. T. Carll. 1962. Mycotoxinoses. *Advances in Veterinary Science*. 7: 272 – 382.
- Forrester, D. J. 1992. *Parasites and diseases of wild mammals in Florida*. Gainesville: University Press of Florida. pp 459.
- Frederick, A. M. 1998. Emerging zoonoses. *Emerging Infectious Diseases Special Issue*. 4: 429 – 435.
- Garcia-Hermoso, D., S. Mathoulin-Pelissier, B. Couprie, O. Ronin, B. Dupont and F. Dromer. 1997. DNA typing suggests pigeon droppings as a source of pathogenic *Cryptococcus neoformans* serotype D. *Journal of Clinical Microbiology*. 35: 2683 – 2685.
- Gilman, J. C. 1959. *A manual of soil fungi*. (2nd Ed.) Great Britain. pp 214 – 234.
- Gitter, M. and P. K. C. Austwick. 1962. Keratomycosis. *Journal of Applied Microbiology*. 179: 602 – 608.
- Goh, K. J., C. T. Tan, N. K. Chew, P. S. K. Tan, A. Kamarulzaman, S. A. Sarji, K. T. Wong, B. J. J. Abdullah, K. B. Chua and S. K. Lam. 2000. Clinical features of Nipah virus encephalitis among pig farmers in Malaysia. *New England Journal of Medicine*. 342: 1229 – 1235.
- Halloran, P. O. 1955. A bibliography of references to diseases in wild mammals and birds. *Journal of Veterinary Research*. 16: 1 – 465.
- Halpin, K., P. L. Young, H. E. Field and J. S. Mackenzie. 2000. Isolation of Hendra virus from pteropid bats: A natural reservoir of Hendra virus. *Journal of General Virology*. 81: 1927 – 1932.
- Hilde, K., M. K. Anne and H. Kjell. 2004. Wildlife as Source of Zoonotic Infections. *Emerging Infectious Diseases*. 10: 2067 – 2072.
- Hooper, P. R., A. Lunt, H. Gould, A. Samaratunga, L. Hyatt, B. Gleeson, C. Rodwell, S. Rupprecht, Smith and P. Murray. 1997. A new lyssavirus – the first endemic rabies-like virus recognized in Australia. *Bulletin Institute of Pasteur*. 95: 209 – 218.
- Jaya Seelan, S. S. and M. Sepiah. 2005. Morphological and physiological characteristics of *Apergillus* spp. on different media, carbon sources and macroelements. 27th Symposium of the Malaysian Society for Microbiology, 24 – 27 November 2005. Grand Plaza Parkroyal, Penang. (Proceeding paper).
- Johara, M., H. Field, A. Rashdi, C. Morissy, B. Van der Heide, P. Rota, A. Adzhar, J. White, P. Daniels, A. Jamaluddin and T. Ksiazek. 2001. Nipah virus infection in bats (Order Chiroptera) in Peninsular Malaysia. *Emerging Infectious Diseases*. 7: 439 – 441.
- Kaliner, G. and J. E. Cooper. 1973. Dual infection of an African fish eagle with acid-fast Bacilli and an *Aspergillus* sp. *Journal of Wildlife Diseases*. 9: 51 – 55.
- Khan, M. M. 1992. *Mamalia Semenanjung Malaysia*. Department of Wildlife and National Park, Malaysia.
- Kim, G. R., Y. T. Lee and C. H. Park. 1994. A new natural reservoir of Hantavirus: Isolation of Hantaviruses from lung tissue of bats. *Achieves of Virology*. 134: 85 – 95.
- Kingston, T., B. L. Lim and A. Zubaid. 2006. *Bats of Krau Wildlife Reserve*. Universiti Kebangsaan Malaysia.
- Koilraj, J. A. and G. Marimuthu. 1999. Fungal diversity inside caves of Southern India. *Current Science*. 75: 1111 – 1113.
- Krause, R. M. 1994. Dynamics of emergence. *Journal of Infectious Diseases*. 170: 265 – 271.

- Krause, R. M. 1992.** The origins of plagues: Old and new. *Science*. 257: 1073 – 1078.
- Kuthubutheen, A. J. and J. Webster. 1986.** Water availability and the coprophilous fungus succession. *Transactions of the British Mycological Society*. 86(1): 63 – 76.
- Lederberge, J., R. E. Shope and S. C. J. Oakes. 1992.** *Emerging Infections: Microbial Threats to Health in the United States*. Washington DC: Institute of Medicine, National Academy Press.
- Leotta, G. A., J. A. Pare, L. Sigler, D. Montalti, G. Vigo, M. Petrucci and E. H. Reinoso. 2002.** *Thelebolus microsporus* Mycelial Mats in the Trachea of Wild Brown Skua (*Catharacta antartica lonnbergi*) and South Polar Skua (*C. maccormicki*) Carcasses. *Journal of Wildlife Diseases*. 38(2): 443 – 447.
- Longmire, J. L., M. Maltbie and R. J. Baker. 1997.** Use of "lysis buffer" in DNA isolation and its implication for museum collections. *Occasional Papers of the Museum of Texas Tech University, Lubbock*. 163: 1 – 3.
- Lyon, G. M., A. V. Bravo, A. Espino, M. D. Lindsley, R. E. Gutierrez, I. Rodriguez, A. Corella, F. Carrillo, M. M. McNeil, D. W. Warnock and R. A. Hajjeh. 2004.** Histoplasmosis associated with exploring a bat-inhabited cave in Costa Rica. *American Journal of Tropical Medicine and Hygiene*. 70: 438 – 442.
- Mancianti, F., W. Mignone and R. Papini. 1997.** Keratinophilic Fungi from Coats of Wild Boars in Italy. *Journal of Wildlife Diseases*. 33(2): 340 – 342.
- Mattsson, R., P. D. Haemig and B. Olsen. 1999.** Feral pigeons as carriers of *Cryptococcus laurentii*, *Cryptococcus uniguttulatus* and *Debaryomyces hansenii*. *Medical Mycology*. 37: 367 – 369.
- Monteros, A. E., L. Carrasco, J. M. King and H. E. Jensen. 1999.** Nasal zygomycosis and pulmonary aspergillosis in an American bison. *Journal of Wildlife Diseases*. 35(4): 790 – 795.
- Moraes, A. M. L., M. Corrado, V. L. Holanda, G. L. Costa, M. Ziccardi, R. Lourenco-de-Oliveira and P. C. Oliveira. 2001.** *Aspergillus* from Brazilian Mosquitoes-I. Genera *Aedeo* and *Culex* from Rio De Janeiro State. *Mycotaxon*. 28: 413 – 422.
- Morse, S. S. 1993.** Examining the origins of emerging viruses. In: Morse, S. S. (Ed.). *Emerging Viruses*. New York: Oxford University Press. pp 10 – 28.
- Noah, D. L., C. L. Drenzek, J. S. Smith, J. W. Krebs, L. Orciari, J. Shaddock, D. Sanderlin, S. Whitfield, M. Fekadu, J. G. Olson, C. E. Rupprecht and J. E. Childs. 1998.** Epidemiology of human rabies in the United States, 1980 to 1996. *Annals of Internal Medicine*. 128: 922 – 930.
- Paez, A., C. Nunez, C. Garcia and J. Boshell. 2003.** Molecular epidemiology of rabies enzootics in Colombia: Evidence for human and dog rabies associated with bats. *Journal of General Virology*. 84: 795 – 802.
- Pane, J., C. M. Francis and K. Phillips. 1998.** *A field guide to the mammals of Borneo*. Sabah Society and World Wildlife Fund: Kota Kinabalu.
- Peden, W. M. and J. L. Richard. 1985.** Mycotic Pneumonia and Meningoencephalitis due to *Aspergillus terreus* in a Neonatal Snow Leopard (*Panthera uncia*). *Journal of Wildlife Diseases*. 12: 301 – 305.
- Philbey, A. W., P. Kirkland, A. Ross, R. Davis, A. Gleeson, P. Daniels, A. Gould and A. Hyatt. 1998.** An apparently new virus (family paramyxoviridae) infections for pigs, humans and fruit bats. *Emerging Infectious Diseases*. 4: 269 – 271.
- Ramirez, R., G. W. Robertstad, L. R. Hutchinson and J. Chavez. 1976.** Mycotic flora in the lower digestive tract of feral pigeons (*Columba livia*) in the El Paso, Texas Area. *Journal of Wildlife Diseases*. 12: 83 – 85.
- Raper, K. B. and D. I. Fennell. 1965.** *The genus Aspergillus*. Baltimore: Williams and Wilkins. pp 686.

- Raper, K. B. and D. I. Fennell. 1977. *The genus Aspergillus*. Baltimore: Williams and Wilkins. pp 686.
- Richardson, L. R., S. Wilkes, J. Godwin and K. R. Pierce. 1962. Effect of moldy diet and moldy soybean meal on the growth of chicks and poults. *Journal of Nutrition*. 78: 301 – 306.
- Rupprecht, C. E., K. Stohr and C. Meredith. 2000. Rabbits. pp 3 – 37. In: Williams, E. S. and I. K. Barker. (Eds.). *Infectious Diseases of Wild Mammals*. (3rd Ed.). Iowa: Iowa State University Press.
- Samson, R. A. and J. I. Pitt. 1990. *Modern Concepts in Penicillium and Aspergillus Classification*. New York and London: Plenum Press.
- Sepiah, M. 1985. Fungi associated with selected species of fruit trees in Malaysia. PhD thesis. Universiti Malaya.
- Silva-Vergara, M. L., E. C. Bento, D. F. Costa, T. F. Costa, C. T. B. Santos and L. Ramirez. 2005. Attempts to isolate *Paracoccidioides brasiliensis* from bats captured in Minas Gerais State of Brazil. IX International on Paracoccidioidomycosis – Aguas de Lindoia, S. Paulo, Brazil. *Revista do Instituto de Medicina Tropical de Sao Paulo*. 47 (Suppl. 14).
- Speare, R., A. D. Thomas, P. O Shea and W. A. Shipton. 1994. *Mucor amphibiorum* in the toad, *Bufo marinus*, in Australia. *Journal of Wildlife Diseases*. 30(3): 399 – 407.
- Takashi, S., K. Ken, M. Koichi, U. Kensaku, S. Takashi, K. Katsuhiko, N. Masakazu and U. Yoshimasa. 2005. *Trichosporon* Species Isolated from Guano Samples Obtained from Bat-Inhabited Caves in Japan. *Journal of Applied and Environmental Microbiology*. 71(11): 7626 – 7629.
- Taylor, L. H., S. M. Latham and M. E. Woolhouse. 2001. Risk factors for human disease emergence. *Biological Sciences*. 356: 983 – 989.
- Tham V. L. and D. A. Purcell. 1974. Fungal Nephritis in a grey-headed albatross. *Journal of Wildlife Diseases*. 10: 306 – 309.
- Trevino, G. S. 1972. Cephalosporiosis in three caimans. *Journal of Wildlife Diseases*. 8: 384 – 388.
- Valdez, H. and R. A. Salata. 1999. Bat-associated histoplasmosis in returning travelers: Case presentation and description of a cluster. *Journal of Travel Medicine*. 6: 258 – 260.
- Wobeser, G. A. 1994. *Investigation and Management of Diseases in Wild Animals*. New York: Plenum Press. pp 265.
- Yamamoto, Y., S. Kohno, H. Koga, H. Kakeya, K. Tomono, M. Kaku, T. Yamazaki, M. Arisawa and K. Hara. 1995. Random amplified polymorphic DNA analysis of clinically and environmentally isolated *Cryptococcus neoformans* in Nagasaki. *Journal of Clinical Microbiology*. 33: 3328 – 3332.
- Chong, H. T., S. R. Kunjapan, T. Thayaparan, J. M. G. Tong, V. Petharumam, M. R. Jusoh and C. T. Tan. 2000. Nipah encephalitis outbreak in Malaysia, clinical features in patients from Seremban. *Neurological Journal of Southeast Asia*. 5: 61 – 67.
- Chua, P. K. B., J. E. Corkill, P. S. Hooi, S. C. Cheng, C. Winstanley and C. A. Hart. 2005. Isolation of *Waddlia malaysiensis*, a novel intracellular bacterium, from fruit bat (*Eonycteris spelea*). *Emerging Infectious Diseases*. 11: 2.
- Chua, K. B., C. L. Koh, P. S. Hooi, K. F. Wee, J. H. Khong and B. H. Chu. 2002a. Isolation of Nipah virus from Malaysian Island flying foxes. *Microbes and Infection*. 4: 145 – 151.
- Chua, K. B., L. F. Wang, S. K. Lam and B. T. Eaton. 2002b. Full length genome sequence of Tioman virus, a novel paramyxovirus in the genus *Rubulavirus* isolated from fruit bats in Malaysia. *Archives in Virology*. 147: 1323 – 1348.
- Chua, K. B., K. J. Goh, K. T. Wong, A. Kamarulzaman, P. S. K. Tan, T. G. Ksiazek, S. R. Zaki, G. Paul, S. K. Lam and C. T. Tan. 1999. Fatal encephalitis due to the Nipah virus among pig-farmers in Malaysia. *The Lancet*. 354: 1257 – 1259.

- Conen, P. E., G. R. Walker, J. A. Turner and P. Field. 1962. Invasive primary aspergillosis of the lung with cerebral metastasis and complete recovery. *Diseases of Chest*. 42: 88–94.
- Coon, N. C. and Locke, L. N. 1968. Aspergillosis in a bald eagle, *Haliaeetus leucocephalus*. *Bulletin of Wildlife Disease Association*. 4: 51.
- Corbet, G. B. and Hill, J. E. 1992. *The mammals of the Indomalayan region: A systematic review*. Oxford: Oxford University Press.
- Cork, S. C., M. R. Alley, A. C. Johnstone and P. H. G. Stockdale. 1999. Aspergillosis and other causes of mortality in the stitch bird in New Zealand. *Journal of Wildlife Diseases*. 35(3): 481–486.
- Cubbon, B. D. 1970. *The Science of Speleology*. London: Academic Press. pp 423.
- Daszak, P., A. A. Cunningham and Hyatt, A. D. 2000. Emerging infectious diseases of wildlife: Threats to biodiversity and human health. *Science*. 287: 443–449.
- Dobson, A. and J. Foufopoulos. 2001. Emerging infectious pathogens of wildlife. *Philosophical Transactions of the Royal Society of London*.
- Ells, T. V., J. Stanton, A. Strieby, P. Daszak, A. D. Hyatt and C. Brown. 2003. Use of immunohistochemistry to diagnose chytridiomycosis in dyeing poison dart frogs *dendrobates tinctorius*. *Journal of Wildlife Diseases*. 30(3): 742–745.
- Erkens, K., M. Lademann, K. Tinteln, M. Lafrenz, U. Kaben and E. C. Reisinger. 2002. Histoplasmosis group disease in bat researchers returning from Cuba. *Deutsche Medizinische Wochenschrift*. 127: 21–25.
- Fooks, A. R., C. Finnegan, N. Johnson, K. Mansfield, L. McElhinney and P. Manser. 2002. Human case of EL type 2 following exposure to bats in Angus, Scotland. *Veterinary Medicine*. 151: 679.
- Forgacs, J. 1962. *Mycotoxicoses in animal and human health*. Proceedings of United States Live Stock Sanitary Association, 66th Annual Meeting. pp 426–448.
- Forgacs, J. and W. T. Carll. 1962. Mycotoxicoses. *Advances in Veterinary Science*. 7: 273–382.
- Forrester, D. J. 1992. *Parasites and diseases of wild mammals in Florida*. Gainesville: University Press of Florida. pp 459.
- Frederick, A. M. 1998. Emerging zoonoses. *Emerging Infectious Diseases Special Issue*. 4: 429–435.
- Garcia-Hermoso, D., S. Mathoulin-Pelissier, B. Couprie, O. Ronin, B. Dupont and F. Dromer. 1997. DNA typing suggests pigeon droppings as a source of pathogenic *Cryptococcus neoformans* serotype D. *Journal of Clinical Microbiology*. 35: 2683–2685.
- Gilman, J. C. 1959. *A mammal of soil fungi*. (2nd Ed.). Great Britain. pp 214–234.
- Gitter, M. and P. K. C. Austwick. 1962. Keratomycosis. *Journal of Applied Microbiology*. 179: 602–608.
- Goh, K. J., C. T. tan, N. K. Chew, P. S. K. Tan, A. Kamarulzaman, S. A. Sarji, K. T. Wong, B. J. J. Abdullah, K. B. Chua and S. K. Lam. 2000. Clinical features of Nipah virus encephalitis among pig farmers in Malaysia. *New England Journal of Medicine*. 342: 1229–1235.
- Halloran, P. O. 1955. A bibliography of references to diseases in wild mammals and birds. *Journal of Veterinary Research*. 16: 1–465.
- Halpin, K., P. L. Young, H. E. Field and J. S. Mackenzie. 2000. Isolation of Hendra virus from pteropid bats: A natural reservoir of Hendra virus. *Journal of General Virology*. 81: 1927–1932.
- Hilde, K., M. K. Anne and H. Kjell. 2004. Wildlife as Source of Zoonotic Infections. *Emerging Infectious Diseases*. 10: 2067–2072.
- Hooper, P. R., A. Lunt, H. Gould, A. Samaratunga, L. Hyatt, B. Gleeson, C. Rodwell, S. Rupprecht, Smith and P. Murray. 1997. A new lyssavirus – the first endemic rabies-like virus recognized in Australia. *Bulletin Institute of Pasteur*. 95: 209–218.

- Jaya Seelan, S. S. and M. Sepiah. 2005. Morphological and physiological characteristics of *Aspergillus* spp. On different media, carbon sources and macroelements. 27th Symposium of the Malaysian Society for Microbiology, 24 – 27 November 2005. Grand Plaza Parkroyal, Penang. (Proceeding paper).
- Johara, M., H. Field, A. Rashdi, C. Morissy, B. Van der Heide, P. Rota, A. Adzhar, J. White, P. Daniels, A. Jamaluddin and T. Ksiazek. 2001. Nipah virus infection in bats (Order Chiroptera) in Peninsular Malaysia. *Emerging Infectious Diseases*. 7: 439–441.
- Kaliner, G. and J. E. Cooper. 1973. Dual infection of an African fish eagle with acid-fast Bacilli and an *Aspergillus* sp. *Journal of Wildlife Diseases*. 9: 51–55.
- Khan, M. M. 1992. *Mamalia Semenanjung Malaysia*. Department of Wildlife and National Park, Malaysia.
- Kim, G. R., Y. T. Lee and C. H. Park. 1994. A new natural reservoir of Hantavirus: Isolation of Hantaviruses from lung tissue of bats. *Achieves of Virology*. 134: 85–95.
- Kingston, T., B. L. Lim and A. Zubaid. 2006. *Bats of Krau Wildlife Reserve*. UKM.
- Koilraj, J. A. and G. Marimuthu. 1999. Fungal diversity inside caves of Southern India. *Current Science*. 75: 1111–1113.
- Krause, R. M. 1994. Dynamics of emergence. *Journal of Infectious Diseases*. 170: 265–271.
- Krause, R. M. 1992. The origins of plagues: Old and new. *Science*. 257: 1073–1078.
- Kuthubutheen, A. J. and J. Webster. 1986. Water availability and the coprophilous fungus succession. *Transactions of the British Mycological Society*. 86(1): 63–76.
- Lederberge, J., R. E. Shope and S. C. J. Oakes. 1992. *Emerging Infections: Microbial Threats to Health in the United States*. Institute of Medicine. Washington DC: National Academy Press.
- Leotta, G. A., J. A., Pare, L. Sigler, D. Montalti, G. Vigo, M. Petrucci and E. H. Reinoso. 2002. *Thebolus microspores* Mycelial Mats in the Trachea of Wild Brown Skua (*Catharacta antarctica lonnbergi*) and South Polar Skua (*C. maccormicki*) Carcasses. *Journal of Wildlife Diseases*. 38(2): 443–447.
- Longmire, J. L., M. maltbie and R. J. Baker. 1997. Use of “lysis buffer” in DNA isolation and its implication for museum collections. *Occasional Papers of the Museum of texas Tech University, Lubbock*. 163: 1–3.
- Lyon, G. M., A. V. Bravo, A. Espino, M. D. Lindsley, R. E. Gutierrez, I. Rodriguez, A. Corella, F. Carrillo, M. M. McNeil, D. W. Warnock and R. A. Hajjeh. 2004. Histoplasmosis associated with exploring a bat-inhabited cave in Costa Rica. *American Journal of Tropical Medicine and Hygiene*. 70: 438–442.
- Mancianti, F., W. Mignone and R. Papini. 1997. Keratinophilic Fungi from Coats of Wild Boars in Italy. *Journal of Wildlife Diseases*. 33(2): 340–342.
- Mattsson, R., P. D. Haemig and B. Olsen. 1999. Feral pigeons as carriers of *Cryptococcus laurentii*, *Cryptococcus uniguttulatus* and *Debaryomyces hansenii*. *Medical Mycology*. 37: 367–369.
- Monteros, A. E., L. Carrasco, J. M. King and H. E. Janssen. 1999. Nasal zygomycosis and pulmonary aspergillosis in an American bison. *Journal of Wildlife Diseases*. 35(4): 790–795.
- Moraes, A. M. L., M. Corrado, V. L. Holanda, G. L. Costa, M. Ziccardi, R. Lourenco-de-Oliveira and P. C. Oliveira. 2001. *Aspergillus* from Brazilian Mosquitoes-I. Genera *Aedeia* and *Culex* from Rio de Janeiro State. *Mycotaxon*. 28: 413–422.
- Morse, S. S. 1993. Examining the origins of emerging viruses. In: Morse, S. S. (Ed.). *Emerging Viruses*. New York: Oxford University Press.

- Noah, D. L., C. L. Drenzek, J. S. Smith, J. W. Krebs, L. Orciari, J. Shaddock, D. Sanderlin, S. Whitfield, M. Fekadu, J. G. Olson, C. E. Rupprecht and J. E. Childs. 1998. Epidemiology of human rabies in the United States, 1980 to 1996. *Annals of Internal Medicine*. 128: 922–930.
- Paez A., C. Nunez, C. Garcia and J. Boshell. 2003. Molecular epidemiology of rabies enzootics in Colombia: Evidence for human and dog rabies associated with bats. *Journal of General Virology*. 84: 795–802.
- Payne, J., C. M. Francis and K. Phillips. 1998. *A field guide to the mammals of Borneo*. Sabah Society and World Wildlife Fund: Kota Kinabalu.
- Peden, W. M. and J. L. Richard. 1985. Mycotic Pneumonia and Meningoencephalitis due to *Aspergillus terreus* in a Neonatal Snow Leopard (*Panthera uncia*). *Journal of Wildlife Diseases*. 12: 301–305.
- Philbey, A. W., P., Kirkland, A. Ross, R. Davis, A. Gleeson, P. Daniels, A. Gould and A. Hyatt. 1998. An apparently new virus (family paramyxoviridae) infections for pigs, humans and fruit bats. *Emerging Infectious Diseases*. 4: 269–271.
- Ramirez, R., G. W. Robertstad, L. R. Hutchinson and J. Chavez. 1976. Mycotic flora in the lower digestive tract of feral pigeons (*Columba livia*) in the El Paso, Texas Area. *Journal of Wildlife Diseases*. 12: 83–85.
- Raper, K. B. and D. I. Fennell. 1977. *The genus Aspergillus*. Baltimore: Williams and Wilkins. pp 686.
- Richardson, L. R., S. Wilkes, J. Godwin and K. R. Pierce. 1962. Effect of moldy diet and moldy soybean meal on the growth of chicks and poults. *Journal of Nutrition*. 78: 301–306.
- Rupprecht, C. E., K. Stohr and C. Meredith. 2000. Rabbits. pp 3–37. In: Williams, E. S. and I. K. Barker. (Eds.). *Infectious diseases of wild mammals*. (3rd Ed.). Iowa: Iowa State University Press.
- Samson, R. A. and J. I. Pitt. 1990. *Modern concepts in Penicillium and Aspergillus classification*. New York and London: Plenum Press.
- Sepiah, M. 1985. Fungi associated with selected species of fruit trees in Malaysia. PhD thesis. Universiti Malaya.
- Silva-Vergara, M. L., E. C. Bento, D. F. Costa, T. F. Costa, C. T. B. Santos and L. Ramirez. 2005. Attempts to isolate *Paracoccidioides brasiliensis* from bats captured in Minas Gerais State of Brazil. IX International on Paracoccidioidomycosis-Aguas de Lindoia, S. Paulo, Brazil. *Revista do Instituto de Medicina Tropical de Sao Paulo*. 47 (Suppl. 14).
- Speare, R., A. D. Thomas, P. Oshea and W. A. Shipton. 1994. *Mucor amphibiorum* in toad, *Bufo marinus*, in Australia. *Journal of Wildlife Diseases*. 30(3): 399–407.
- Takashi, S., K. Ken, M. Koichi, U. Kensaku, S. Takashi, K. Katsuhiko, N. Masakazu and U. Yoshimasa. 2005. *Trichosporon* Species Isolated from Guano Samples Obtained from Bat-Inhabited Caves in Japan. *Journal of Applied and Environmental Microbiology*. 71(11) 7626–7629.
- Taylor, L. H., S. M. Latham and M. E. Woolhouse. 2001. Risk factors for human disease emergence. *Biological Sciences*. 356: 983–989.
- Tham, V. L. and D. A. Purcell. 1974. Fungal Nephritis in a grey-headed albatross. *Journal of Wildlife Diseases*. 10: 306–309.
- Trevino, G. S. 1972. Cephalosporiosis in three caimans. *Journal of Wildlife Diseases*. 8: 384–388.
- Valdez, H. and R. A. Salata. 1999. *Investigation and management of diseases in wild animals*. New York: Plenum Press. pp 265.
- Yamamoto, Y., S. Kohno, H. Koga, H. Kakeya, K. Tomono, M. Kaku, T. Yamazaki, M. Arisawa and K. Hara. 1995. Random amplified polymorphic DNA analysis of clinically and environmentally isolated *Cryptococcus neoformans* in Nagasaki. *Journal of Clinical Microbiology*. 33: 3328–3332.