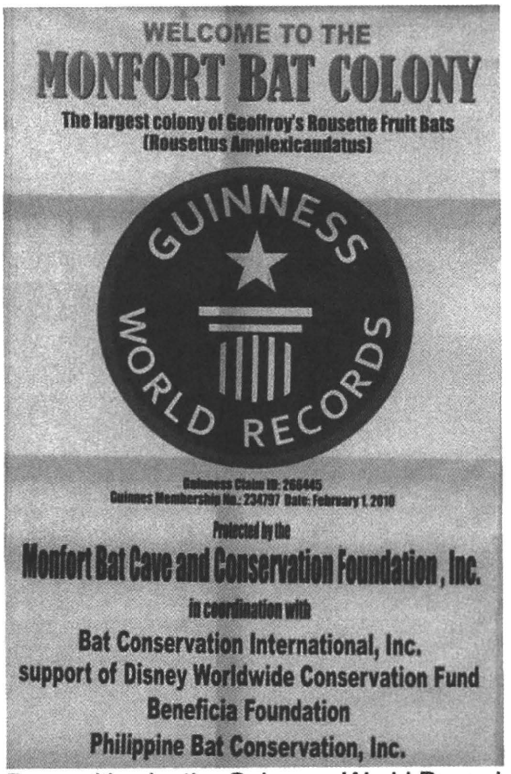
inside wall of the cave but even found hanging along the rim of the hole and some were even hanging on the roots of the trees. Because of the natural contour of the land with a gradual uphill ascent cold air enters the first hole, flows to the other three holes and finally the now humid and hot air tends to go up and exits through the last hole. This last hole is at the end and serves as a chimney. If one puts his hand near the opening, warm humid air can be felt. With a population of approximately 2.5 million bats the body heat produced by the bats alone plus the chemical decomposition of guano can easily increase the ambient temperature and humidity inside the cave. Without a natural way of ventilation the bats can easily suffer from hyperthermia that could lead to a massive die-off. In the past years guano was regularly harvested from the cave for agricultural fertilizer. However, because of the big possibility of disturbing the bats this practice of guano harvesting was stopped. Another big question to ask is the reason for the increase in the population of bats. Careful observation showed that the bats do not have a specific breeding season but rather a continuous cycle of reproduction. Scientists had documented a continuous sexual activity all throughout the day and night. In fact because of this observation there is a popular joke that the Monfort cave is the biggest brothel in town. Sexual activity is non-stop and copulation was observed even right after birth. The Rousette Fruit Bats (*Rousettus amplexicaudatus*) is truly a polygamous species practicing both polygyny (one male to several females) and polyandry (one female to several males). Pups (baby bats) are observed throughout the year at varying ages of development.

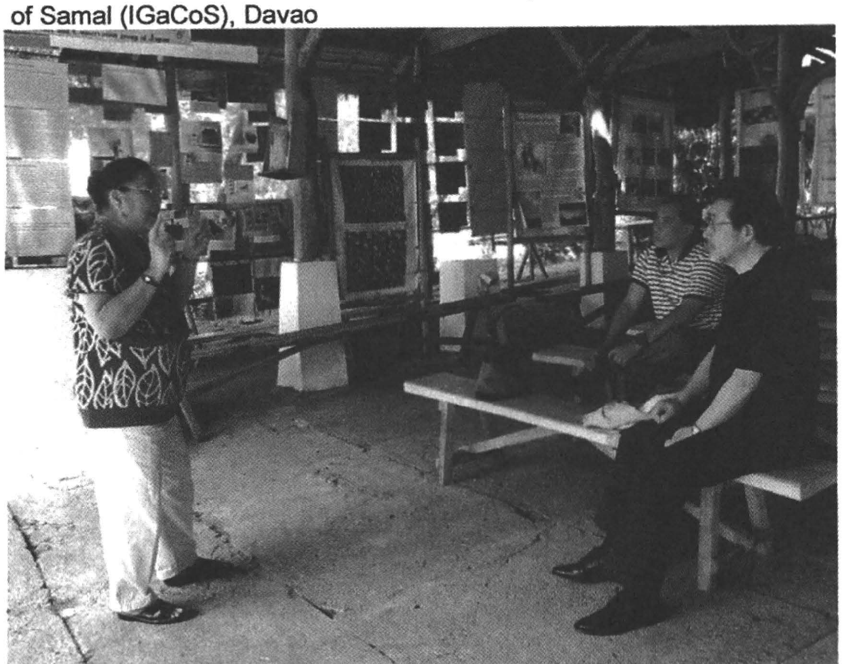
It is not a joke to feed 2.5 million bats and a conservative estimate showed that if one bat will just eat 10 grams of fruit a night they would have consumed 25 tons of fruits. Bats are crepuscular (active during dusk and dawn) to nocturnal (active during the night) and it was observed that feeding time is staggered within the colony. Even the direction of flight out of the cave is divided into different waves and directions going to the mainland of Davao where more fruits can be found. The return flight to the cave is also staggered with a definite regular schedule. If you are a social drinker do not assume that only people have the monopoly on drinking and getting drunk in the process. Animals also enjoy the alcoholic content of fruits. A species of monkey was observed in Africa to protect a favourite tree when it starts to bear fruits. The troop of monkeys which guards the tree within the territory has the sole bragging right to claim the tree. Sentinels are posted in strategic position to prevent untimely and early harvest by other members of the troop. Once the fruit has ripened and starts to ferment producing alcohol in the process then the party begins. It was observed that the monkeys really prefer the fermenting fruits because of high alcohol content. After eating the fermented fruit the monkeys really get drunk with swaying movement and loss of muscle control with some even falling to the ground in a very tipsy manner. In the Monfort cave the Rousette Fruit Bats (*Rousettus amplexicaudatus*) were also observed to practice and seem to enjoy this practice of eating fermented fruits. This batch of drunkards, usually males, is the last group to return to the cave after feeding in the mainland. Even from far away one can notice that they are returning because of the loud sound they produce. Indeed they are a raucous noisy band of drunkard bats. Another tree, in the Sudano-Sahelian range of West Africa known as Marula (*Sclerocarya birrea*) which in English is known as jelly plum, cat thorn, morula, cider tree, is a favorite of many animals especially when the fruits become over ripe and fall to the ground. The fermenting fruits attract different animals including both mammals and birds. After gorging on the fermented fruits the animals like wild pigs, wildebeest (gnu), elephants, baboons, giraffe, ostrich, and meerkats, go home tipsy and drunk.

For us veterinarians our concern is the possibility of the Rousette Fruit Bats (*Rousettus amplexicaudatus*) carrying potential pathogens for both humans and animals. In Africa it has been proven that the Egyptian rousette bats (*Rousettus aegyptiacus*) are carriers of the dreaded Ebola virus. In the past studies in the Philippines conducted by different research institutes the link between pigs and bats with regards to the transmission of REBOV has not been clearly established. The presence of a monospecific population of Rousette Fruit Bats (*Rousettus amplexicaudatus*) in a dense population in Monfort cave is a very challenging situation and can provide valuable data in the elucidation of the link between fruit bats and REBOV. Hopefully in the future we will be given the permit and opportunity to examine and analyze bat specimens from this cave so that we can have a clear understanding of the ecological relationship between the host and its pathogens

I

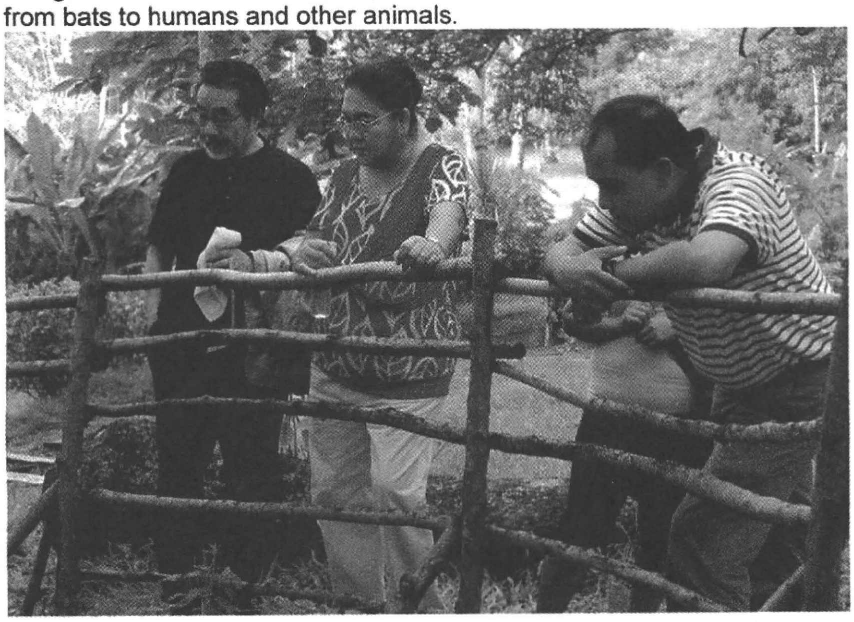


Recognition by the Guinness World Records for the largest colony of Geoffroy's Rousette Fruit Bats (Rousettus amplexicaudatus) in the Monfort Cave located in the Island Garden City of Samal (IGaCoS), Davao

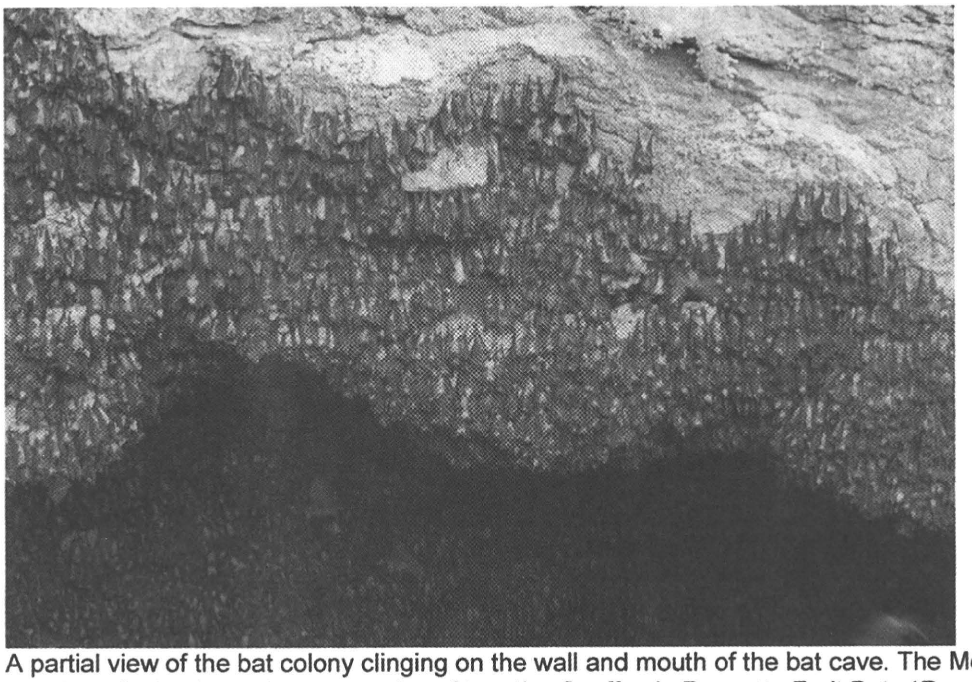


The owner of the land, Ms. Norma Monfort, where the bat colony is located, giving an orientation lecture about the features of the bat colony.

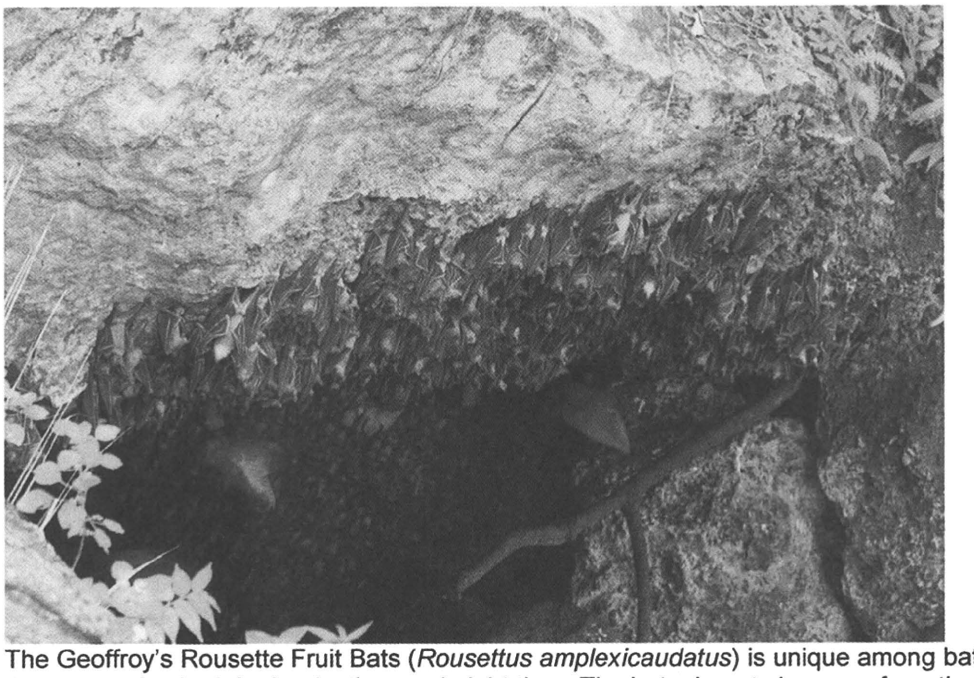
Dr. Yasuhiro Yoshikawa (left) with Dr. Joseph S. Masangkay (right) and Ms. Norma Monfort (center). Both Dr. Yoshikawa and Dr. Masangkay are doing collaborative research on possible diseases that can be transmitted from bats to humans and other animals.



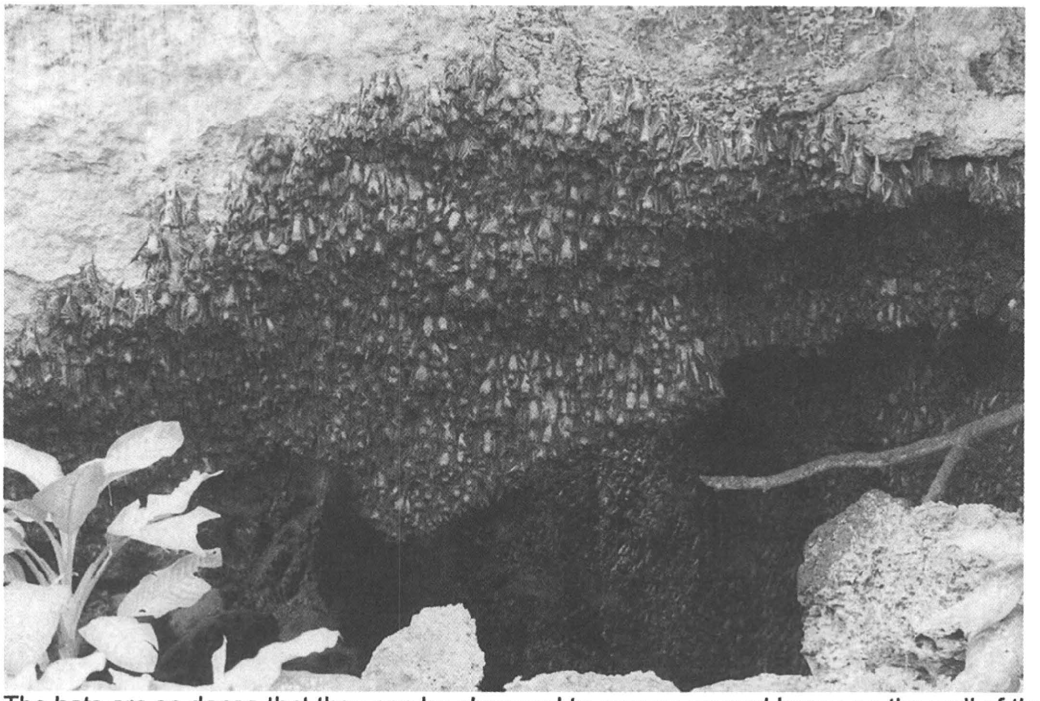
The visiting scientists were guided by the owner, Ms. Norma Monfort in observing the bats. On the right is Dr. Roberto P. Puentespina, Jr. who is a practicing veterinarian in Davao City. Dr. Puentespina's family is the owner of Malagos Garden Resort in Davao. Both Ms. Monfort and Dr. Puentespina are concerned with ecological protection and wildlife conservation doing several collaborative projects with different NGOs and private foundations. Take note of the barrier fence constructed around the hole of the bat caves to prevent people from getting very near the bats and disturbing them. The fence also prevents other stray animals like dogs, goats and pigs in getting inside the cave. Nursing mother bats when disturbed might drop their pups leading to sure death of the pups because of the very thick guano on the floor of the cave.



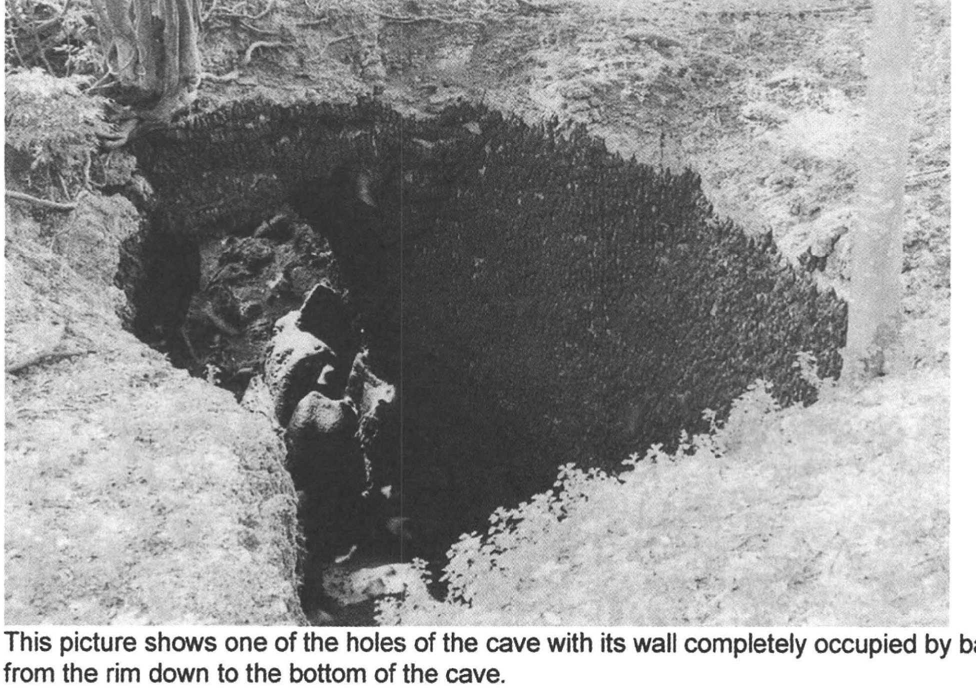
A partial view of the bat colony clinging on the wall and mouth of the bat cave. The Monfort Bat Cave Is unique for having only one species of bat, the Geoffroy's Rousette Fruit Bats (*Rousettus amplexicaudatus*). Most often a bat cave will contain more than one species of bats. This bat colony was first estimated to have 1.8 million bats but lately the population has grown to almost 2.5 million making it to the Guinness World Record.



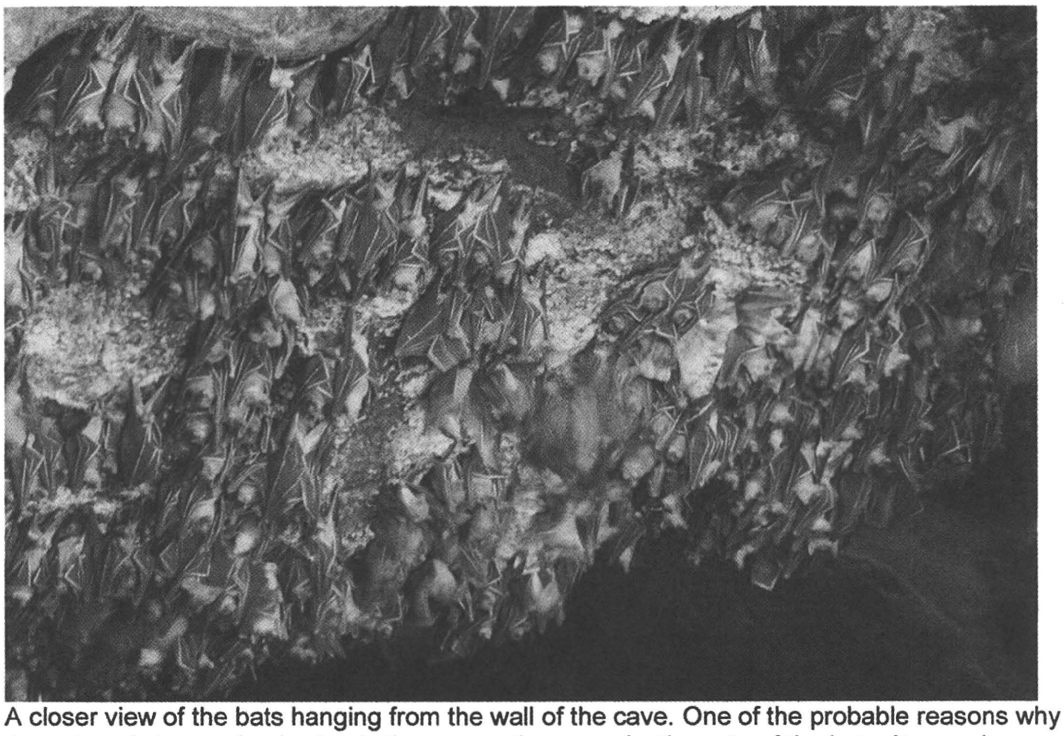
The Geoffroy's Rousette Fruit Bats (*Rousettus amplexicaudatus*) is unique among bats because they are active both in the daytime and night time. The bats do not shy away from the light as you can see in this picture with many of them hanging on the outside rim of the cave and some even flying (the blurred portion of the picture because of the movement of the wings).



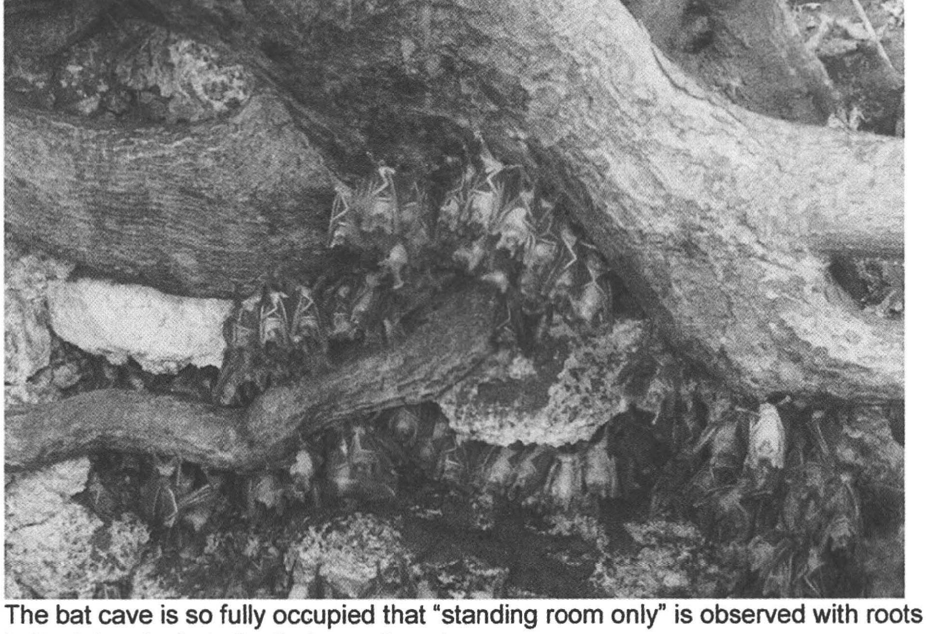
The bats are so dense that they can be observed to occupy several layers on the wall of the cave. A conservative estimate pegged the density of the bat as approximately 640 individual bats per square meter. Because of the big size of the colony it also produces a lot of guano which was harvested before for agricultural fertilizer. Lately, however, because of the disturbance during the harvesting of guano, this practice is now totally stopped.



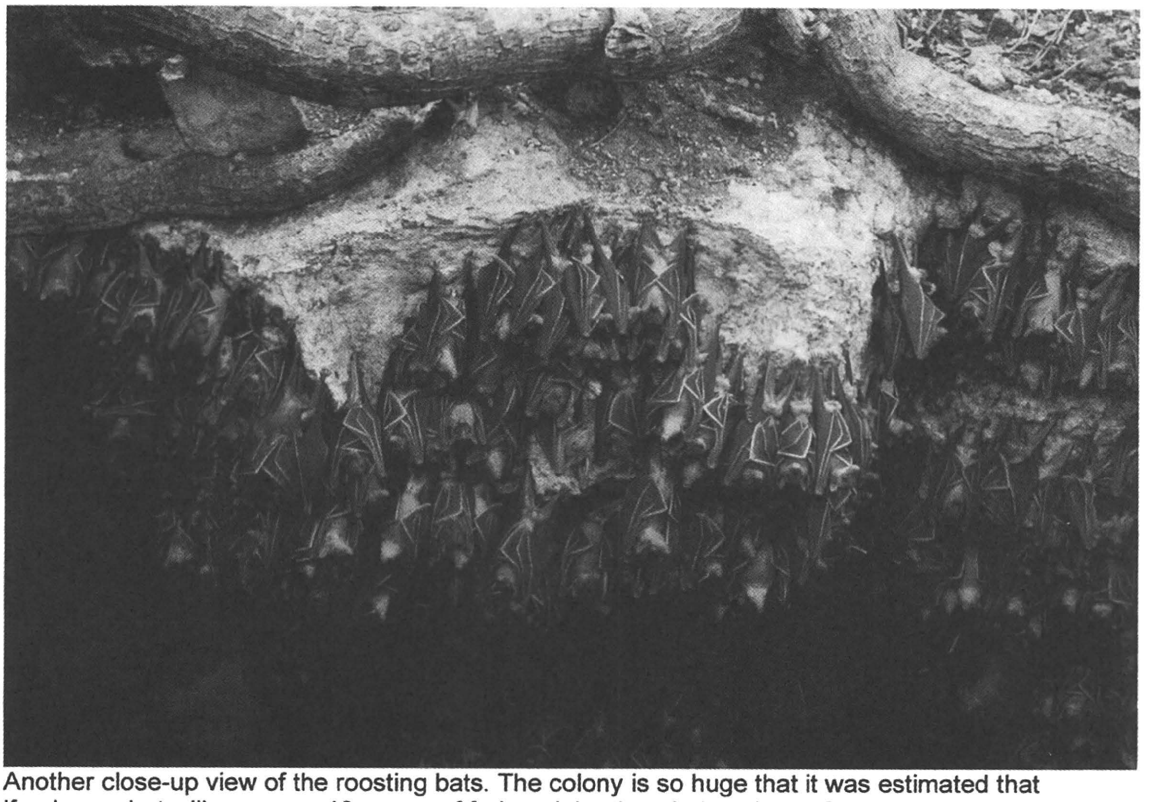
This picture shows one of the holes of the cave with its wall completely occupied by bats from the rim down to the bottom of the cave.



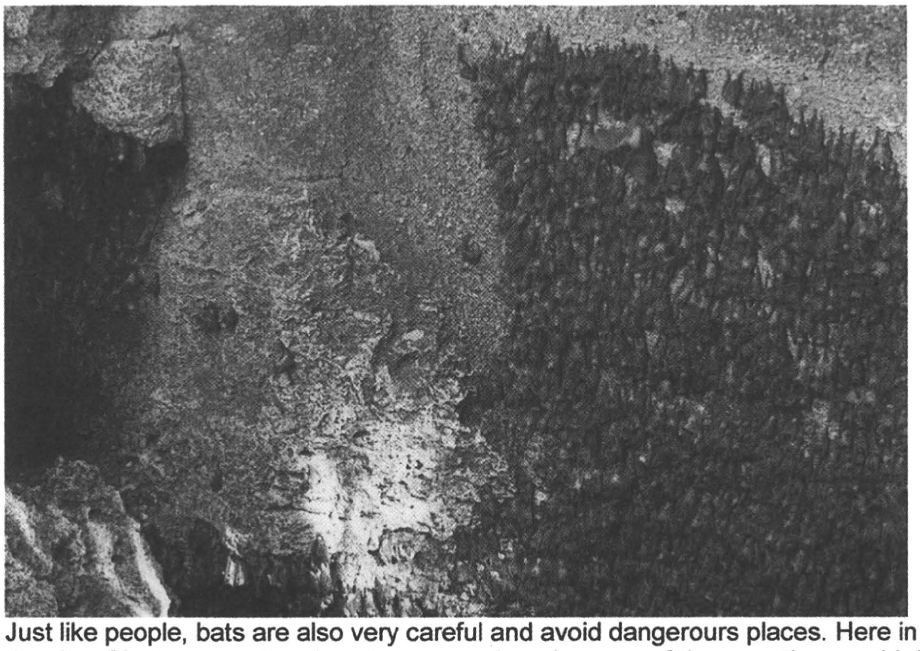
A closer view of the bats hanging from the wall of the cave. One of the probable reasons why the colony is increasing in size is the very active reproductive rate of the bats. It was observed that there is no clearly-delineated breeding season. Sexual activity is continuous 24/7 with bats copulating both in the day and night. The bats also practice true polygamy with one male copulating with several females (polygyny) and one female copulating with several males (polyandry). Even pregnant females are being copulated by males and sometimes even immediately after birth copulation was also noted. There is a famous joke circulating in the island that the Monfort cave is the largest and most active brothel in town.



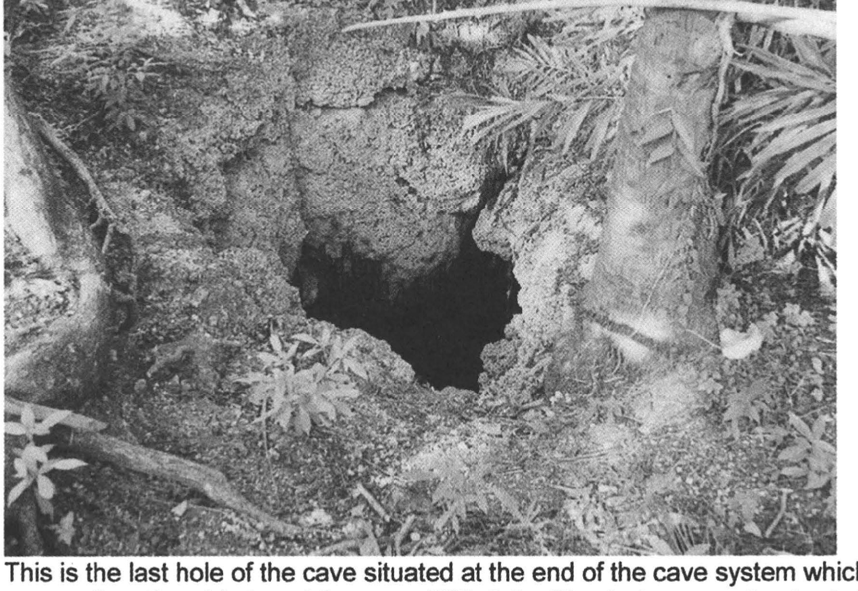
The bat cave is so fully occupied that "standing room only" is observed with roots of trees being taken by bats for their roosting place.



Another close-up view of the roosting bats. The colony is so huge that it was estimated that if only one bat will consume 10 grams of fruit a night, the whole colony of 2.5 million bats would have consumed a total of 25 tons of fruits a night. However, this observation should always be taken with a grain of salt because it might be construed that the fruit bats are destructive to fruit trees which is not true. It was observed that the fruit bats only eat the over ripe fruits which can also benefit the fruit farmers because in effect the bats help in cleaning the farm by getting rid of the over ripe fruits that can serve as breeding place for harmful insects like the destructive fruit fly. Fruit bats also help in the dispersal of seeds to assure continuous natural planting of trees that can help mitigate global warming.



Just like people, bats are also very careful and avoid dangerours places. Here in spite of the heavy density of bats occupying almost every nook and cranny of the cave they avoid dangerous places like the waterway passage as shown in the center of this picture. During heavy rain this waterway passage becomes a "falls" and any bat caught in the deluge will be washed down and surely end up in the bottom of the cave and get mired in the thick guano guaranteeing a sure death.



This is the last hole of the cave situated at the end of the cave system which has the highest elevation among the other 4 holes of the cave. With 2.5 million bats generating body heat plus the chemical decomposition of guano the bats would have suffered from hyperthermia because of the high temperature and humidity that can cause a massive die-off. The land area where the cave is situated has a natural system of HVAC (heating, ventilation and air conditioning). Because of the lay of the land with its gradual ascent uphill, the first hole at the lowest portion of the cave sucks in cold air coming from the sea, goes through the whole length of the cave, gathers heat and moisture and finally exits through the last hole situated at the end of the cave system funnelling out the warm humid air. In effect this last hole serves as a natural "chimney" for the cave to dissipate heat. If one puts his hand near the vent, warm air can be felt.

Title of Research Project: FRUIT BATS (*Rousettus amplexicaudatus*) AS POTENTIAL CARRIERS OF Reston Ebola Virus (REBOV) IN MONKEYS

Period covered: 2010 July 1 to 2011 March 31

Purpose of research: To determine the presence of REBOV antibodies in FRUIT BATS (*Rousettus amplexicaudatus)* in the Philippines

Research Team composed of research staff from the following:

1. University of the Philippines Los Baños, College of Veterinary Medicine headed by Dr. Joseph S. Masangkay
2. University of Tokyo, Graduate School of Agricultural and Life Sciences headed by Dr. Yasuhiro Yoshikawa.
3. University of the Philippines Los Baños, Museum of Natural History headed by Phillip Alviola

RATIONALE: One of the dreaded zoonotic diseases is Ebola Hemorrhagic Fever caused by different strains of the virus Ebola. The name was originated from the Ebola River Valley in the Democratic Republic of the Congo (formerly Zaire), near the site of the first recognized outbreak in 1976. The disease is not widely known until 1989 when outbreaks among monkeys were reported in the United States. There are many strains of the virus but the most lethal is the Zaire ebolavirus (ZEBOV). Other strains include Sudan ebolavirus (SEBOV), Côte d'Ivoire ebolavirus (CIEBOV), Bundibugyo ebolavirus, and Reston ebolavirus (REBOV). Our main interest is REBOV since it is not fatal to humans but very fatal in monkeys. ***Reston ebolavirus*** was first recognized in the United States among monkeys in 1989 in Reston, Virginia followed by another report in Alice, Texas. Both of these infection were reported in cynomologus monkeys (*Macaca fascicularis*) imported from the Philippines. Similar findings in imported monkeys was also reported in an import facility in Italy in 1994. From monkeys the virus jumped species and reported among pigs in the Philippines in 2008.

It is for this reason that the team wanted to know if bats, particularly *Rousettus amplexicaudatus* found within the vicinity of monkey breeding facilities are positive for the presence of antibodies for REBOV. This is important because the mere presence of antibodies in an animal does not mean that the animals have active infection. Findings from this research can help government policy-makers in formulating news bulletins and news report to the public to avoid undue problem and panic which can drastically affect the pig industry if an alarm is sounded that will prevent the public from buying and consuming pork and pork by-products.

ACTIVITIES:

1. Preliminary survey to determine the trapping site for bats.
2. Application for a permit from concerned government authorities like the Protected Areas and Wildlife Bureau of the Department of Environment and Natural Resources Regional Office and owner of the identified sites of capture and submission of research proposal for approval
3. Field work proper for capturing of bat specimen – two sites from July 7 to 13, 2011. The two identified sites are:
    a. Tanay, Rizal – Simian Conservation Breeding and Research Center (SICONBREC)
    b Sto. Tomas Batangas – INA Research Philippines (INARP)
4. Laboratory work to examine, analyze and collect blood and tissue samples from the captured bats
5. Population ocular survey of the biggest *Rousettus amplexicaudatus* bat colony in the world located in a bat cave in Samal Island, Davao – February 2 -5, 2011.
6. Report writing

ACCOMPLISHMENTS:

Publications:

1. **Masangkay,** J. S, Yoshikawa, Y. and Alviola, Phillip. BATS and MONKEYS. Animal Scene (Vol No. 10 Issue No. 7) September 2010 issue. pp 88-92
2. Yoshikawa, Y. and **Masangkay**, J. 2010. Trace of Reston Ebolavirus-Capture of bats in the Philippines (in Japanese). *Labio 21*. No. 42. Oct. 2010. Pp. 28-31
3. Reyes, A.W.B., Rovira, H.G., **Masangkay**, J.S., Ramirez, T.J., Yoshikawa, Y. and Baticados, W.N. 2011. Polymerase Chain Reaction Assay and Conventional Isolation of *Salmonella* spp.from Philippine Bats. *Acta Scientiae Veterinariae*, 2011. 39 (1): 947.
4. Report for the Monfort Bat Cave will be submitted later

FUTURE PLANS: if we can negotiate for a research activity to be done at the Monfort Bat Cave in Samal, Island we will do some bat specimen collection because the samples we got from the vicinity of the two monkey-breeding facilities only yielded one specimen of *Rousettus amplexicaudatus.* Since the Monfort Cave is composed of only of 1 species of bat, the *Rousettus amplexicaudatus,* there is a big chance that we can get some significant results for the incidence of antibodies for REBOV.

Joseph S. Masangkay, DVM, MS, PhD
Professor

V．業績資料集

野生動物・輸入動物に関する研究G

宇根　有美／麻布大学
佐野　文子／千葉大学
吉川　泰弘／北里大学

J. Comp. Path. 2010, Vol. 143, 150–156
Available online at www.sciencedirect.com
ScienceDirect
ELSEVIER
www.elsevier.com/locate/jcpa

Pathological Changes in Captive Monkeys with Spontaneous Yersiniosis due to Infection by *Yersinia enterocolitica* serovar O8

S. Nakamura*, H. Hayashidani†, T. Iwata†, S. Namai‡ and Y. Une*

* *Laboratory of Veterinary Pathology, Azabu University, Fuchinobe 1-17-71, Sagamihara, Kanagawa 229-8501,* † *Institute of Symbiotic Science and Technology, Tokyo University of Agriculture and Technology and* ‡ *Tobu Zoo, Japan*

Summary

An outbreak of fatal yersiniosis due to infection with *Yersinia enterocolitica* serovar O8 is documented in two species of captive monkey. Five of 50 squirrel monkeys (*Saimiri sciureus*) and one of two agile gibbons (*Hylobates agilis*) died following several days of diarrhoea. Necropsy examination revealed necrotizing enterocolitis and multifocal necrosis or abscesses in various organs. Microscopically, these lesions comprised multifocal necrosis with bacterial colonies, neutrophils and accumulation of nuclear debris. Occasional lesions included macrophages and abscess formation. Immunohistochemically, the bacteria were identified as *Y. enterocolitica* O8. In addition, *Y. enterocolitica* serotype O8 was isolated from animal organs in pure culture. This is the first report of fatal cases of infection with *Y. enterocolitica* serovar O8 in animals.

Keywords: outbreak; squirrel monkey; *Yersinia enterocolitica* serovar O8; yersiniosis

Introduction

Yersiniosis is a zoonotic disease caused by *Yersinia enterocolitica* or *Yersinia pseudotuberculosis* and appears as enteritis and sometimes septicaemia in man and animals. Latent infection by these species occurs in free-living wild mice and wild birds and bacteria are excreted in their faeces. Contaminated food and water are common sources for the introduction of pathogens (Fukushima *et al.*, 1988; Sunahara *et al.*, 2000; Han *et al.*, 2003). These pathogens have a very wide host range and have been detected in more than 110 species of animal worldwide, including mammals, birds and reptiles (Shayegani *et al.*, 1986; Kwaga and Iversen, 1993; Greene, 1998). Infection is almost always latent and there have been sporadic reports of occurrences in animals such as non-human primates, rabbits, guinea pigs, chinchillas, livestock and birds, with the disease appearing as enteritis, mesenteric lymphadenitis, multiple nodules with necrosis, abscess formation and septicaemia (Mair, 1973; Baskin *et al.*, 1977; Harcourt-Brown, 1978; Hanssen, 1982; Parsons, 1991; Seimiya *et al.*, 2005). *Y. enterocolitica* is found more frequently than *Y. pseudotuberculosis*, but actual cases are limited to a few species, such as non-human primates and chinchillas, and the lesions are similar to those of *Y. pseudotuberculosis*.

Y. enterocolitica has over 60 serotypes, but only a limited number of these (i.e. serotypes O3, O4/32, O5/27, O8, O9, O13a, O13b, O18, O20 and O21) are pathogenic in man. Of these the most frequently detected pathogenic serotypes are O3, O5/27, O8 and O9. Serotypes O3, O5/27 and O9 are distributed worldwide and many cases have been reported in man and animals, while serotype O8 is limited to North America and is thus referred to as the North American strain (Schiemann, 1989). Serotype O8 has been isolated mainly from human patients (Bissett, 1976; Winblad, 1979; Shayegani *et al.*, 1983) and a few healthy foxes (*Urocyon cinereoargenteus*) and porcupines (Shayegani *et al.*, 1986) and is supposedly confined to North America. However, isolation of this serotype from human patients and healthy wild rodents has been reported sporadically in Japan since 1990 (Ichinohe *et al.*, 1991; Iinuma *et al.*, 1992;

Correspondence to: Y. Une (e-mail: une@azabu-u.ac.jp).

0021-9975/$ - see front matter
doi:10.1016/j.jcpa.2010.01.017

Hayashidani *et al.*, 1995; Sakai *et al.*, 2005) and it has recently been reported in Europe (Schubert *et al.*, 2003; Rastawicki *et al.*, 2009). However, in animals, fatal or non-fatal cases caused by this serotype have not been reported. Serotypes O3, O5/27 and O9 have relatively low pathogenicity and mainly cause diarrhoea, but serotype O8 is highly pathogenic in man and may cause severe septicaemia (Schiemann *et al.*, 1981). In mice experimentally infected with serotypes O3, O5/27 and O9, infection can be subclinical, but in infection with O8 almost 100% of individuals become moribund with septicaemia (Carter *et al.*, 1973; Maruyama *et al.*, 1979).

Non-human primates appear to be sensitive to *Y. enterocolitica* and *Y. pseudotuberculosis* and many fatal cases of yersiniosis have been reported worldwide (Mair *et al.*, 1970; McClure *et al.*, 1971; Baggs *et al.*, 1976; Poelma *et al.*, 1977; Vandamme *et al.*, 1978; Buhles *et al.*, 1981; MacArthur and Wood, 1983; Skavlen *et al.*, 1985; Plesler and Claros, 1992; Sasaki *et al.*, 1996; Kageyama *et al.*, 2002). In Japan, squirrel monkeys (*Saimiri sciureus*) are reported to be highly sensitive to *Y. pseudotuberculosis*, and outbreaks occur every year in zoos (Iwata *et al.*, 2008). There have also been reports of non-human primate infection with pathogenic *Y. enterocolitica* elsewhere, but no such infections have yet been reported in Japan. This is the first report of animal fatalities due to *Y. enterocolitica* serotype O8. The aim of the present study was to elucidate the pathological features of an outbreak of *Y. enterocolitica* O8 infection in captive monkeys in Japan.

Materials and Methods

Case History

In December 2002, five of 50 squirrel monkeys housed at a zoo in Japan died following several days of diarrhoea. At that point, 14 of the remaining 45 monkeys had diarrhoea. The outbreak was controlled by treatment with antibiotics. However, after the end of the outbreak, in April 2003, one of two agile gibbons (*Hylobates agilis*) showed similar clinical signs and was given antibiotics for 3 days, but the symptoms worsened and the animal died on the fourth day. The squirrel monkeys were fed fruits, such as apples and bananas, and commercial monkey food, and were housed in an indoor-outdoor enclosure. Other monkeys were housed in outdoor cages about 50 m from the squirrel monkey enclosure.

Animals and Pathological Examination

The subjects of this report were five squirrel monkeys and one agile gibbon (Table 1). All squirrel monkeys were juveniles aged less than 1 year (four males and one female). The agile gibbon was a 14-year-old female. A complete necropsy examination was performed on each dead animal (except one squirrel monkey) as soon as possible after death in the Laboratory of Veterinary Pathology of Azabu University. For microscopical examination, specimens of various tissues were fixed in 10% neutral buffered formalin and embedded in paraffin wax. Sections (3 μm) were stained with haematoxylin and eosin (HE) and Gram stain (by the method of Brown—Hopps).

Table 1
Details of animals investigated

Case no.	*Species*	*Date of death*	*Body weight*	*Age*	*Clinical signs*
1	SM 1	8/12/2002	180 g	Juvenile	NA
2	SM 2	22/12/2002	288 g	Juvenile	Diarrhoea, mandibular swelling
3	SM 3	23/12/2002	NA	Juvenile	Diarrhoea
4	SM 4	28/12/2002	272 g	Juvenile	Depression
5	SM 5	29/12/2002	250 g	Juvenile	Mandibular swelling
6	Agile gibbon	4/4/2003	5 kg	Adult	Diarrhoea, depression

SM, squirrel monkey; NA, data not available.

Immunohistochemistry (IHC) was performed using a set of commercial rabbit anti-*Y. enterocolitica* sera specific for O1-2, O3, O5, O8 and O9 and a set of anti-*Y. pseudotuberculosis* sera specific for O1, O2, O3, O4, O5 and O6 (Denka-Seiken Co., Tokyo). Secondary reactions were performed with a peroxidase-conjugated Histofine-Simplestain kit (Simplestain MAX-PO; Nichirei, Tokyo). 3, 3′-diaminobenzidine and H_2O_2 was used to 'visualize' the reaction products. Slides were counterstained with Mayer's haematoxylin.

Bacteriological Culture and Molecular Typing

Bacteriological features and the results of molecular typing of isolates have been described previously (Iwata *et al.*, 2005). Briefly, organs (liver, spleen, lung, intestine and mandibular abscesses) or faecal samples collected from the five dead squirrel monkeys and one dead agile gibbon, and 98 faecal samples (45 from squirrel monkeys, 20 from other monkeys of 18 different species and 33 from black rats [*Rattus rattus*] captured around the monkey cages) were examined for the presence of *Yersinia* spp. by culture on irgasan-novobiocin (IN) agar plates. *Y. enterocolitica* serovar O8 was then isolated based on biochemical characteristics and a slide agglutination test. In addition, isolates were examined using the molecular typing method based on pulsed field gel electrophoresis (PFGE), ribotyping and restriction endonuclease analysis of virulence plasmid DNA (REAP) (Iwata *et al.*, 2005).

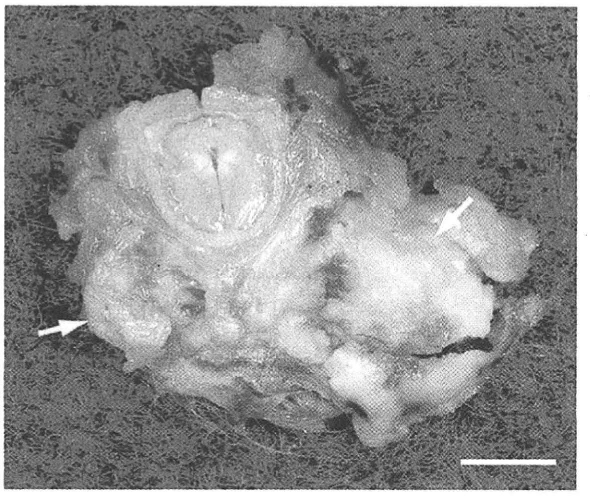


Fig. 1. Cut surface of head and neck area of a squirrel monkey (case 2) after formalin fixation. Lymph nodes are enlarged and abscessed (arrows). Bar, 0.5 cm.

Results

Gross Pathology

Three of the five squirrel monkeys had mandibular swelling with enlarged submandibular lymph nodes. In one severe case a large abscess was present in the mandible, with a fistulous tract draining to the skin of the neck (Fig. 1). Intestinal lesions of varying severity were present in all cases. Adhesive peritonitis with perforation caused by necrotizing enteritis of the small intestine was observed. This was characterized by notable enlargement of Peyer's patches (PPs) and solitary lymphoid nodules (Fig. 2). There was significant enlargement of the mesenteric lymph nodes (MLNs), which often contained abscesses, and intestinal adhesion was also present. Multiple swollen, yellow-white nodules were observed in the spleen and liver. There were also abscesses in the lungs and kidneys.

In the agile gibbon there was peritonitis with marked accumulation of abdominal fluid. Lesions in the gastrointestinal tract were primarily of the ileocaecal area where there was a large abscess with abscessation of the caecal lymph nodes. There were no lesions of the small intestinal PPs. Numerous large abscesses surrounded by multiple smaller yellow-white nodules were present in the liver (Fig. 3). The centres of the abscesses were filled with inflammatory exudate. The spleen was moderately swollen. Other findings included swelling and small white nodules in the gastric lymph nodes and lymph nodes near the kidney. A summary of the pathological findings is given in Table 2.

Histopathology

In the squirrel monkeys, alimentary lesions occurred mainly in the small intestinal PPs and in solitary lymphoid nodules of the caecum and colon. There was also mucosal necrosis with desquamation of the epithelium and congestion and/or haemorrhage and accumulation of nuclear debris. In addition, there were numerous bacterial colonies in the lesions, with marked infiltration by neutrophils and macrophages (Fig. 4). Lymphoid follicles were depleted of lymphocytes and largely replaced by large bacterial colonies and inflammatory infiltration. Similar lesions were present in the MLNs, in the lymph nodes of the neck and in the tonsils (Fig. 5). In severe cases, the lymph nodes were entirely replaced by an abscess. Multiple foci of necrosis with bacterial colonies and occasional abscess formation were seen in many organs, including the liver, spleen, kidneys, lungs, tonsils and lymph nodes.

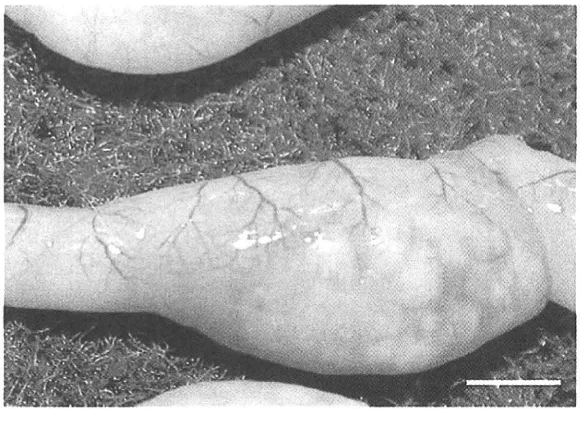


Fig. 2. PP of a squirrel monkey (case 5) showing enlargement and the formation of white nodules. Bar, 0.5 cm.

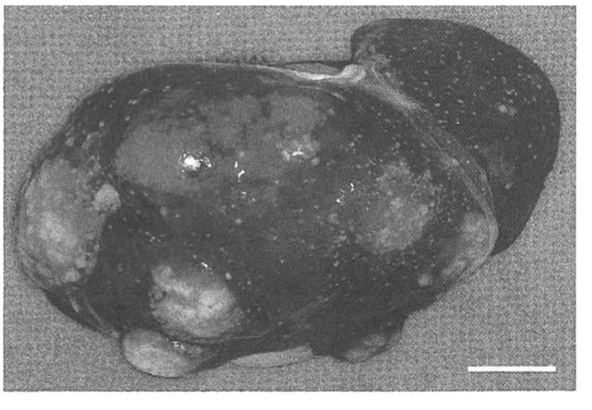


Fig. 3. Liver of the agile gibbon (case 6). There are numerous large abscesses surrounded by multiple small yellow-white nodules. Bar, 2 cm.

Table 2
Summary of pathological findings

Animal	*Gross findings*							*Microscopical findings*									
	Liver	*Spleen*	*Intestine*	*PPs*	*MLNs*	*HN*	*Other*	*Liver*	*Spleen*	*Intestine*	*PPs*	*MLNs*	*Neck*	*Other*	*Isolation*	*IHC*	
SM 1	NA	NA	+ + +	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	+	NA	
SM 2	–	+ +	–	+ + +	+ + +	+ + +	+	+ +	+ +	–	+ +	+ + +	+ + +	+ + +	+	+	
SM 3	–	+	+ + +	NA	+ + +	NA	–	+	+	+ +	NA	+ + +	NA	+	+	+	
SM 4	–	–	–	–	–	+	+	+	+	+	+	–	–	+ + +	+	+	
SM 5	+ +	+ +	–	+	+ + +	+ + +	+	+ +	+ +	–	+	+ + +	+ + +	+ + +	+	+	
Gibbon	+ + +	+	+ +	–	+ + +	–	+	+ + +	+ +	+ + +	–	+ + +	–	+ + +	+	+	

HN, head and neck region; Other, other organs; NA, data not available. Gross findings are scored as no lesion (–), swelling (+), multiple white nodules (+ +), abscess formation (+ + +). Microscopical findings are scored as no lesion (–), neutrophil infiltration (+), focal necrosis with bacterial colonies (+ +), abscess formation (+ + +). Isolation and IHC are either positive (+) or negative (–) for *Y. enterocolitica* O8 by bacterial culture and IHC, respectively.

In the agile gibbon, microscopical lesions were similar to those observed in the squirrel monkeys. There was severe necrosis in all layers of the ileum, colon and caecum, with numerous bacterial colonies. There were also multiple foci of necrosis in the liver, with numerous bacterial colonies and neutrophils, accumulation of nuclear debris and occasional macrophages. Similar lesions were seen in the spleen, lungs and lymph nodes. Hepatocytes had marked accumulation of haemosiderin as confirmed by Perl's Prussian blue staining.

Bacterial colonies were comprised of gram-negative bacilli and were strongly labeled by *Y. enterocolitica* O8 antiserum (Fig. 6), but were negative or only slightly positive for other serotypes (O1-2, O3, O5 and O9) and also for *Y. pseudotuberculosis* (O1, O2, O3, O4, O5 and O6) serotypes (Table 2).

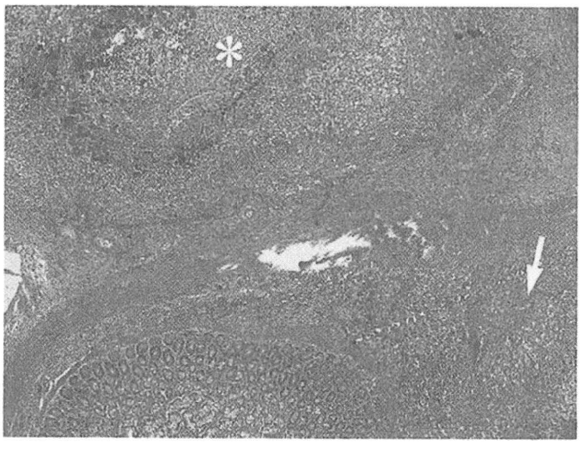


Fig. 4. Caecum from a squirrel monkey (case 5) showing solitary lymphoid nodules (arrow). The caecal lymph node is indicated by the asterisk. Lymphoid follicles of the solitary lymphoid nodules and the caecal lymph node were depleted and replaced by large bacterial colonies and inflammatory cell infiltration. HE. ×40.

Bacteriological Culture and Molecular Typing

Only *Y. enterocolitica* serovar O8 was isolated from the five dead squirrel monkeys and agile gibbon. The same organism and serovar was isolated from 21 of 65 (32.3%) other monkeys and five of 33 (15.2%) black rats. Furthermore, almost all of the isolates were identical by PFGE, ribotyping and REAP analysis. Bacteriological features and the results of molecular typing of isolates have been described previously (Iwata *et al.*, 2005).

Discussion

The five squirrel monkeys and an agile gibbon investigated in the present study exhibited necrotizing enteritis and enlargement of the spleen and liver, accompanied by multifocal necrosis with intralesional gram-negative bacilli. These bacterial colonies were characterized immunohistochemically as *Y. enterocolitica* serovar O8. These lesions are typical of those reported in other monkeys with yersiniosis. The

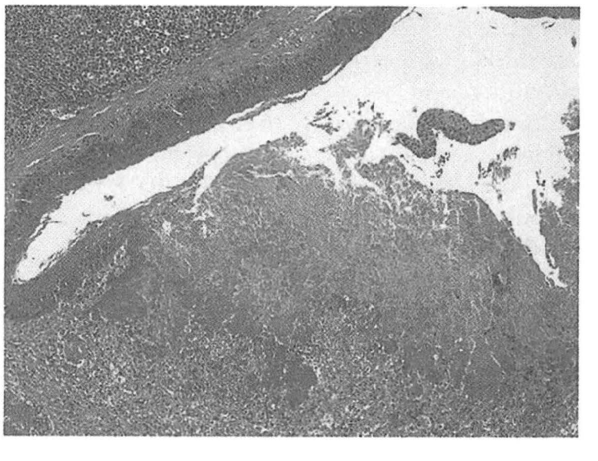


Fig. 5. Tonsil from a squirrel monkey (case 5). Lymphoid follicles are replaced by large bacterial colonies and inflammatory cell infiltration. HE. ×40.

Fig. 6. Liver of the agile gibbon (case 6) showing labelling of bacterial colonies by anti-*Y. enterocolitica* O8 sera. IHC. ×100.

diagnosis of yersiniosis was confirmed by isolation of *Y. enterocolitica* serotype O8 in pure culture from various organs and molecular typing proved these isolates to be identical. Based on these findings, this outbreak was considered to be yersiniosis due to *Yersinia enterocolitica* serotype O8 and death was most likely to be caused by sepsis. Fatal and non-fatal cases of infection with this serotype have only been reported in human patients and have been unknown in animals to date. Therefore, to our knowledge, this report is the first description of fatal yersiniosis due to infection with *Y. enterocolitica* serovar O8 in animals.

Based on the molecular typing of isolates from squirrel monkeys, agile gibbons and black rats, the isolates appeared to have originated from a common source. Given the time interval between the outbreak of yersiniosis in the squirrel monkeys and the occurrence of disease in the agile gibbon, the initial outbreak is thought to have occurred among the squirrel monkeys, with subsequent infection of the agile gibbon. The squirrel monkey cage is distant from those of other monkey species, including the agile gibbon, and is separated from the road on which visitors pass. This suggests that direct transmission from the squirrel monkeys to the agile gibbon was unlikely. Moreover, as the isolates from black rats had the same molecular genotype as those from the monkeys, the black rats may have carried the infection between the squirrel monkeys and other monkeys. Indeed, wild rodents are generally considered to be the source of infection in Japanese patients with yersiniosis due to *Y. enterocolitica* serovar O8 (Iinuma *et al.*, 1992; Hayashidani *et al.*, 1995). However, the origin and route of transmission in *Y. enterocolitica* serovar O8 cases remains unclear, as compared with other pathogenic serotypes such as O3, O5/27 and O9. Similarly, in this case, as the origin or route of transmission of bacteria remains unclear, further epidemiological studies are required.

The pathological findings in the squirrel monkeys were similar to lesions caused in monkeys by infection with *Y. pseudotuberculosis*, although there were several notable differences. In these cases the lesions tended to be suppurative with abscessation. *Y. enterocolitica* O8 is known to induce a wide range of lesions in lymphoid organs, particularly the tonsils and neck lymph nodes, to a greater degree than occurs in infection with *Y. pseudotuberculosis*. In addition, 17 of 50 squirrel monkeys had diarrhoea, whereas in *Y. pseudotuberculosis* infection in squirrel monkeys, diarrhoea is rarely seen and sudden death may occur in the absence of clinical signs. Therefore, the pathogenicity of *Y. enterocolitica* O8 appears to be lower than that of *Y. pseudotuberculosis* and the disease appears to progress more slowly in squirrel monkeys. *Y. pseudotuberculosis* isolated from Far East regions, including Japan, has the *ypm* (*Y. pseudotuberculisis*-derived mitogen) gene encoding the superantigenic toxin, while *Y. enterocolitica* does not carry the *ypm* gene (Yoshino *et al.*, 1995). Superantigen YPM is considered to be the virulence factor associated with a variety of clinical signs in human yersiniosis, including fever, scarlatiniform rash, diarrhoea, vomiting and arthritis (Abe *et al.*, 1993, 1997; Uchiyama *et al.*, 1993; Ueshiba *et al.*, 1998). The difference between the lesions formed by *Y. enterocolitica* O8 and *Y. pseudotuberculosis* in squirrel monkeys may be due to the presence of the *ypm* gene.

There was a difference in the distribution of lesions in the two species of monkey reported here. In the squirrel monkeys, the lesions were seen mainly in small intestinal PPs, but there were no similar lesions in the gibbon. Lesions in the gibbon were focused on the ileocaecal region. Yersiniosis due to *Y. pseudotuberculisis* in apes (including gibbons) and adult people is often characterized by lesions in the ileocaecal region with hepatic abscessation (Skavlen *et al.*, 1985). The gibbon reported here had lesions consistent with previous reports of lesions associated with *Y. pseudotuberculisis* infection. Therefore, host susceptibility to *Yersinia* may differ depending on monkey species.

Pathogenic *Yersinia* of various serotypes have been isolated from human patients and wild animals, livestock and companion animals. Because many outbreaks have been reported to be caused by pathogenic *Yersinia*, particularly in monkey species in zoos, this infection is considered to be important in terms of animal husbandry. The present study reports the first fatal case of yersiniosis due to *Y. enterocolitica* serovar O8, known as the American strain. Serotype O8 is highly virulent when compared with other pathogenic serotypes, including O3, O5/27 and O9. Pathogenic *Yersinia* including *Y. enterocolitica* serovar O8 cause zoonotic diseases and we cannot exclude the possibility of human infection from monkeys, particularly because of the absence of clinical disease. Moreover, infected animals excrete faeces that can contaminate food and the surrounding environment and it is difficult to prevent carriers such as wild rodents from entering animal enclosures. Therefore, from the point of view of public and animal health, extreme caution and additional sanitary precautions are necessary in order to preclude transmission of pathogenic *Yersinia* spp. As there are many questions regarding serotypes and pathogenic relationships in yersiniosis, further detailed pathological and epidemiological studies remain necessary.

Acknowledgment

We are grateful to Professor K. Shirota of Azabu University for his advice.

References

Abe J, Onimaru M, Matsumoto S, Noma S, Baba K *et al.* (1997) Clinical role for a superantigen in *Yersinia pseudotuberculosis* infection. *Journal of Clinical Investigation*, **99**, 1823–1830.

Abe J, Takeda T, Watanabe Y, Nakano H, Kobayashi N *et al.* (1993) Evidence for superantigen production by *Yersinia pseudotuberculosis*. *Journal of Immunology*, **151**, 4183–4185.

Baggs RB, Hunt RD, Garcia FG, Hajema EM, Blake BJ *et al.* (1976) Pseudotuberculosis (*Yersinia enterocolitica*) in the owl monkey (*Aotus trivirgatus*. *Laboratory Animal Science*, **26**, 1079–1083.

Baskin GB, Montali RJ, Bush M, Quan TJ, Smith E (1977) Yersiniosis in captive exotic mammals. *Journal of the American Veterinary Medical Association*, **171**, 908–912.

Bissett ML (1976) *Yersinia enterocolitica* isolates from humans in California, 1968–1975. *Journal of Clinical Microbiology*, **4**, 137–144.

Buhles WC, Vanderlip JE, Russell SW, Alexander NL (1981) *Yersinia pseudotuberculosis* infection: study of an epizootic in squirrel monkeys. *Journal of Clinical Microbiology*, **13**, 519–525.

Carter PB, Varga CF, Keet EE (1973) New strain of *Yersinia enterocolitica* pathogenic for rodents. *Applied Microbiology*, **26**, 1016–1018.

Fukushima H, Gomyoda M, Shiozawa K, Kaneko S, Tsubokura M (1988) *Yersinia pseudotuberculosis* infection contracted through water contaminated by a wild animal. *Journal of Clinical Microbiology*, **26**, 584–585.

Greene CE (1998) Yersinosis. In: *Infectious Diseases of the Dog and Cat*, 2nd Edit., CE Green, Ed., Saunders, Philadelphia, pp. 241–242.

Han TH, Paik IK, Kim SJ (2003) Molecular relatedness between isolates *Yersinia pseudotuberculosis* from a patient and an isolate from mountain spring water. *Journal of Korean Medical Science*, **18**, 425–428.

Hanssen I (1982) *Yersinia pseudotuberculosis* infection in captive black grouse (*Tetrao lyrurus*) and willow ptarmigan (*Lagopus l. lagopus*. *Acta Veterinaria Scandinavica*, **23**, 622–623.

Harcourt-Brown NH (1978) *Yersinia pseudotuberculosis* infection in birds. *Veterinary Record*, **102**, 315.

Hayashidani H, Ohtomo Y, Toyokawa Y, Saito M, Kaneko K *et al.* (1995) Potential sources of sporadic human infection with *Yersinia enterocolitica* serovar O8 in Aomori Prefecture, Japan. *Journal of Clinical Microbiology*, **33**, 1253–1257.

Ichinohe H, Yoshioka M, Fukushima H, Kaneko S, Maruyama T (1991) First isolation of *Yersinia enterocolitica* serotype O8 in Japan. *Journal of Clinical Microbiology*, **29**, 846–847.

Iinuma Y, Hayashidani H, Kaneko K, Ogawa M, Hamasaki S (1992) Isolation of *Yersinia enterocolitica* serovar O8 from free-living small rodents in Japan. *Journal of Clinical Microbiology*, **30**, 240–242.

Iwata T, Une Y, Okatani AT, Kaneko S, Namai S *et al.* (2005) *Yersinia enterocolitica* serovar O8 infection in breeding monkeys in Japan. *Microbiology and Immunology*, **49**, 1–7.

Iwata T, Une Y, Okatani AT, Kato Y, Nakadai A *et al.* (2008) Virulence characteristics of *Yersinia pseudotuberculosis* isolated from breeding monkeys in Japan. *Veterinary Microbiology*, **129**, 404–409.

Kageyama T, Ogasawara A, Fukushima R, Narita Y, Miwa N *et al.* (2002) *Yersinia pseudotuberculosis* infection in breeding monkeys: detection and analysis of strain diversity by PCR. *Journal of Medical Primatology*, **31**, 129–135.

Kwaga J, Iversen JO (1993) Isolation of *Yersinia enterocolitica* (0:5,27 biotype 2) from a common garter snake. *Journal of Wildlife Diseases*, **29**, 127–129.

MacArthur JA, Wood M (1983) Yersiniosis in a breeding unit of *Macaca fascicularis* (cynomolgus monkeys). *Laboratory Animals*, **17**, 151–155.

Mair NS (1973) Yersiniosis in wildlife and its public health implications. *Journal of Wildlife Diseases*, **9**, 64–71.

Mair NS, White GD, Schubert FK, Harbourne JK (1970) *Yersinia enterocolitica* infection in the bush-baby (Galago). *Veterinary Record*, **86**, 69–71.

Maruyama T, Une T, Zen-Yoji H (1979) Observations on the correlation between pathogenicity and serovars of *Yersinia enterocolitica* by the assay applying cell culture system and experimental mouse infection. *Contributions to Microbiology and Immunology*, **5**, 317–323.

McClure HM, Weaver RE, Kaufmann AF (1971) Pseudotuberculosis in nonhuman primates: infection with organisms of the *Yersinia enterocolitica* group. *Laboratory Animal Science*, **21**, 376–382.

Parsons R (1991) Pseudotuberculosis at the Zoological Society of London (1981 to 1987). *Veterinary Record*, **128**, 130–132.

Plesler R, Claros M (1992) A spontaneous *Yersinia pseudotuberculosis* infection in a monkey colony. *Journal of Veterinary Medicine Series B*, **39**, 201–208.

Poelma FG, Borst GH, Zwart P (1977) *Yersinia enterocolitica* infections in non-human primates. *Acta zoologica et pathologica Antverpiensia*, **69**, 3–9.