

An investigation of wild guars Death in Kui Buri National Park, Thailand, 2013

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Abstract

Wild gaur is an endangered wildlife species since its numbers have drastically declined due to over-hunting and habitat destruction. In 17 December 2013, four gaurs' carcasses were found in Kunshorn Forest Plantation, Kui Buri National Park, Thailand. The cause of death was doubtful. The investigation team of National Institute of Animal Health (NIAH), Prachuabkhirikhan Provincial Livestock Office, Department of National Park, Wildlife and Plant Conservation and Faculty of Veterinary Medicine, Mahidol University performed investigation to verify causes of the death and provide recommendation to prevent the problem in future. Eighteen carcasses that were found until 29 December 2013 were examined and sampled. Bacterial viral, parasitic infection and toxic substances were differentiated by laboratory diagnosis at NIAH. Sixteen out of 18 carcasses had been autopsied, and 100 samples of macerated tissue, ruminal content and soil samples were collected. The results revealed only *Clostridium novyi* type B 50% (8/16), Foot and mouth disease 6.25% (1/16) and *Anaplasma marginalae* 12.5% (2/16) from tissue samples, and nitrate 100% (16/16) from all ruminal content samples. However, no fatal infectious disease was found because of poor quality samples. Base on these findings, the laboratory results cannot suggest causative agent of the death. Health condition of alive wild animals in Kunshorn Forest Plantation and nearby domestic livestock should be monitored. After investigation, all carcasses were buried and disinfected with lime and the biosecurity system was implemented especially in the entrance of the area. Urea fertilizer was prohibited to prevent nitrate toxin are the urgently preventive scheme.

Keywords: Wild Guar, Kui Buri National Park, *Clostridium novyi*, *Anaplasma marginalae*,
Foot and mouth disease, Nitrate

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การสอบสวนหาสาเหตุการตายของกระทิงป่าที่อุทยานแห่งชาติกุยบุรีในปี พ.ศ. 2556

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บทคัดย่อ

กระทิงป่าจัดเป็นสัตว์ป่ากลุ่มเสี่ยงใกล้สูญพันธุ์ชนิดหนึ่งเนื่องจากการถูกล่า และแหล่งที่อยู่อาศัยถูกทำลาย ทั้งนี้ เมื่อวันที่ 17 ธันวาคม 2556 มีรายงานการพบซากกระทิงป่าซึ่งตายโดยไม่ทราบสาเหตุ อยู่ในพื้นที่โครงการอนุรักษ์ฟื้นฟูสภาพป่าสงวนแห่งชาติกุยบุรี อันเนื่องมาจากพระราชดำริ (โครงการกุยชอ) อุทยานแห่งชาติกุยบุรี จังหวัดประจวบคีรีขันธ์ จำนวน 4 ตัว และพบซากกระทิงเพิ่มขึ้นรวมเป็นจำนวน 18 ตัว เมื่อนับถึงวันที่ 29 ธันวาคม 2556 ทีมสอบสวนโรคจากสถาบันสุขภาพสัตว์แห่งชาติ สำนักงานปศุสัตว์จังหวัดประจวบคีรีขันธ์ กรมอุทยานแห่งชาติ สัตว์ป่าและพันธุ์พืช และคณะสัตวแพทยศาสตร์ มหาวิทยาลัยมหิดล ได้ร่วมกันลงพื้นที่สอบสวนหาสาเหตุการตายของกระทิง และให้คำแนะนำเพื่อป้องกันการเกิดปัญหาดังกล่าว โดยทำการผ่าซากกระทิง 16 ตัว และเลือกเก็บตัวอย่างเนื้อเยื่อที่มีการเน่าสลายน้อยที่สุด หนุ่ในกระเพาะ และดินใต้ซาก รวมจำนวน 100 ตัวอย่าง ส่งห้องปฏิบัติการทางแบคทีเรีย ไวรัส ปรสิตร และสารพิษ ผลการตรวจพบเชื้อแบคทีเรีย *Clostridium novyi* type B 50% (8/16) เชื้อไวรัสโรคปากและเท้าเปื่อย 6.25% (1/16) และพยาธิเม็ดเลือดแดง *Anaplasma marginalae* 12.5% (2/16) จากตัวอย่างเนื้อเยื่อ และพบสารไนเตรตจากหนุ่ในกระเพาะทุกตัวอย่าง (16/16) ไม่พบเชื้อก่อโรคร้ายแรงซึ่งอาจเนื่องมาจากคุณภาพตัวอย่าง จากผลการตรวจวิเคราะห์ดังกล่าว ไม่สามารถสรุปสาเหตุการตายได้ ดังนั้น จึงควรเฝ้าระวังสุขภาพสัตว์ป่าที่เหลืออยู่และปศุสัตว์ที่อยู่รอบอุทยาน และทำลายซากกระทิงโดยการโรยด้วยปูนขาวและชุดหลุมฝัง พร้อมทำการสร้างบ่อน้ำยาฆ่าเชื้อบริเวณทางเข้าเขตอุทยาน นอกจากนี้ ยังแนะนำให้ปฎิบัติในแปลงหนุ่เพื่อป้องกันการปัญหาพิษจากสารไนเตรต

คำสำคัญ: กระทิงป่า อุทยานแห่งชาติกุยบุรี *Clostridium novyi*, *Anaplasma marginalae*, โรคปากและเท้าเปื่อย สารไนเตรต

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Introduction

The wild gaur belongs to *B. gaurus* species, Bos Genus and Bovidae Family. It is the largest animal species in this genus. Members of this genus contain both domestic animals such as cattle, buffalo, sheep, goat and wild animals such as *Bubalus amee*, *Bos gaurus*, *B. javanicus*, *B. sauveli*, *Capricornis milneedwardsii*, *C. sumatraensis* and *Naemorhaedus griseus*. Previously, three subspecies of gaur including *Bos gaurus gaurus*, *B. g. readei* and *B. g. hubbacki* have been identified in South Asia and Southeast Asia. However, Groves [1] concluded that there were only two subspecies: *Bos gaurus gaurus*, found in India and Nepal, and *Bos gaurus laosiensis*, found in Myanmar, Lao PDR, Viet Nam, Cambodia, Thailand, and West Malaysia. Nationally, gaur lives in small herds of eight to eleven individuals and led by an old adult female, the matriarch. Adult males may be solitary and join the herd in breeding season. Like other ruminants, gaur normally grazes and browses young green grasses and leaves. It can eat fruits, twigs, bark of various woody species, as well as coarse dry grasses, and bamboo [2].

In 1994, Department of National Park, Wildlife and Plant Conservation (DNP) estimated that there were 920 wild guars in the wildlife protected area in Thailand [3]. The population of wild guars is in decreasing trend. It was mainly a result of decreasing forestry area and illegal hunting. Moreover, some infectious diseases had also considered harmful to wild guars such as Foot and Mouth Disease (FMD) (4), Rinderpest [5], and non-specific diarrhea, non-specific fever, conjunctivitis, skin disease and mastitis [6]. However, there are no reports of any infectious diseases outbreak in wild guar in Thailand.

DNP office reported four carcasses of wild guars found in the Kunshorn Forest Plantation Project, Kui Buri National Park, Thailand on December 17, 2013. Local investigators estimated period of the death to be around late of November. The carcasses showed no sign of bullet wounds or other injuries. Ruminal infectious diseases such as FMD, hemorrhagic septicemia and blood parasites had been concerned because these three major diseases are harmful to many species of livestock. However, there was no report of these diseases in ruminants in Kuiburi district since January 2013. All carcasses had remained in the scenes at that moment pending for further examination. Because wild gaur is the endangered animal species in Thailand and the cause of death was not clear, investigation team comprising staffs from National Institute of Animal Health (NIAH), Prachuabkhirikhan Provincial Livestock Office

(PPLO), DNP and Veterinary Medicine, Mahidol University (Vet MU) visited this area and conducted investigation to identify the cause of the death, implement control measures to prevent death in other gaurs and provide recommendation to prevent the problem in future.

Materials and methods

Since the first notification, 18 carcasses had been found consequently till December 29, 2013. Investigation team visited the area, performed autopsy, collected information i.e. geographical location, environment, age and sex of gaur. Other unusual marks especially smell were noted during the observation. Because most of the carcasses were rotten and some were bitten by wild animals, gaur's age and sex were determined by some characteristic of carcasses. To determine animal age, small size carcass with brown hair coat was younger than six months old, while adult wild gaur are characterized by size, black hair coat with white stocking on all four legs, and number of annular band and sharpness of horn [2]. Gender was determined by their horn characteristic. Male's horn has wider curve than female's horn.

Most of the carcasses were rotten when staffs found them. Thus, only mild macerated tissues from the rotten carcasses were samples. Samples from environment i.e. soil sample under the carcass, ruminal content, visceral organs, the remaining lymph nodes, bone marrow, and ruminal contents were collected for laboratory testing. All samples were sent to test for all possible pathogenic microorganisms at National Institute of Animal Health, Department of Livestock Development, Thailand. Identification of pathogenic microorganisms was carried out by Polymerase Chain Reaction technique (PCR). The diagnosed pathogenic microorganism tests were blood parasitic diseases (*Anaplasma* spp., *Babesia* spp., *Trypanosoma* spp.), viral diseases (Malignant Catarrhal Fever, Mobilivirus infection, Rinderpest, Swine vesicular disease, Peste des petis, Foot and Mouth Disease, Bunyavirus infection, West Nile virus infection, Ndumu virus infection, Babankivirus infection, Batavirus infection, Sindbisvirus infection, Rift Valley Fever virus infection, Chikungunyavirus infection and Coronavirus infection) and bacterial infectious diseases (Anthrax, Clostridium Infection and Hemorrhagic Septicemia).

All ruminal content samples were submitted for toxic substances testing such as chemical agents (carbamate, organophosphate and organochlorine), heavy metal (cadmium, cobalt, chromium, iron and lead), mineral (manganese and molybdenum), arsenic, cyanide, warfarin and nitrate. Moreover, soil under rumen position of each carcass was also tested for

toxic substances which may flow through from rumen content to soil by gravity in macerated carcasses. All samples were extracted and GC-MS (Gas Chromatography–Mass Spectrometry) was applied to analyze carbamate, organophosphate and organochlorine, while ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometry) was applied to analyze heavy metal, mineral arsenic, cyanide, warfarin and Spectrophotometer was applied to test nitrate.

Results

A total of 18 wild guar carcasses scattered around thirteen square kilometers of the dense forest in the Kunshorn Forest Plantation, Kui Buri National Park, Thailand as shown in the figure 1. Appearance of the carcasses was varied form complete carcass (8 carcasses), bitten by wild animals (7 carcasses), bitten by wild animals and scattered organs (2 carcasses) and only bone remaining (1 carcass). There were 12 males and 6 females. Most of the dead gaurs were aged higher than 10 years (16 gaurs). Others were 3 months old (1 gaur) and 7 years old (1 gaur).

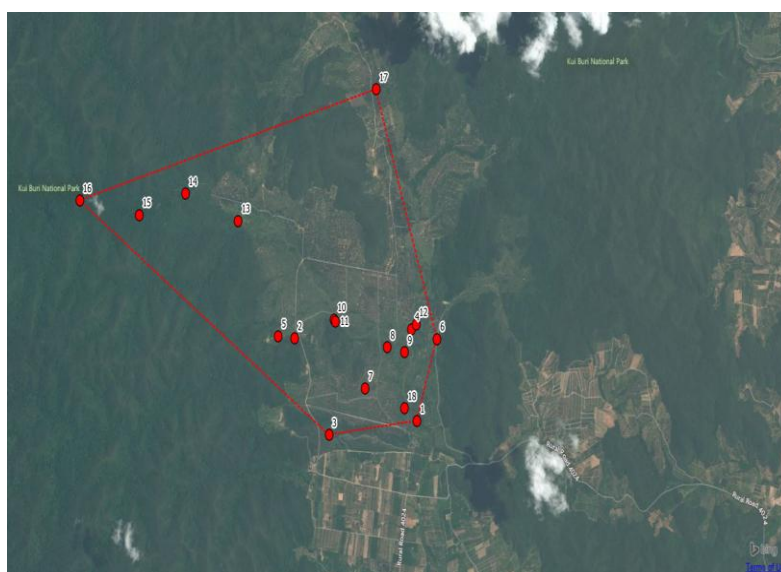


Figure 1. Geographical distribution of gaur carcass Kunshorn Forest Plantation Area, Kui Buri National Park, Thailand, 2013

There was no sign of bullet wounds or other injury in all carcasses. No observable lesion was identified in all complete carcasses (8 carcasses). Ten out of 16 carcasses presented ammonia smell in various degrees. Posture of gaur during death was both sternal and lateral recumbency. Most of the carcasses (16 carcasses) had full ruminal content. The condition of all carcasses and their physical appearance were shown in table 1.

Table 1. The general information and physical appearance of gaur carcasses in Kunshorn Forest Plantation Area, Kui Buri National Park, Thailand, 2013.

Number of carcass	Estimated age	Sex	Date found (Dec 2013)	Physical appearance	Ruminal content
1	15 years	M	02	Complete carcass	Full
2	7 years	F	09	Bitten by wild animal	Full
3	20 years	M	13	Complete carcass	Full
4	>10 years	M	17	Bitten by wild animal	Full
5	10 years	F	18	Bitten by wild animal	Full
6	3 months	M	19	Bitten by wild animal	Full
7	15 years	M	19	Bitten by wild animal	Full
8	13 years	F	20	Complete carcass	Full
9	15 years	M	22	Complete carcass	Full
10	15 years	M	22	Complete carcass	Full
11	15 years	M	22	Complete carcass	Full
12	13 years	M	22	Only bone remained	NA
13	12 years	M	23	Bitten by wild animal and scatter	Full
14	15 years	F	24	Bitten by wild animal and scatter	Full
15	13 years	F	24	Bitten by wild animal	Full
16	16 years	F	24	Bitten by wild animal	Full
17	11 years	M	26	Complete carcass, found in swamp	Full
18	16 years	M	29	Buried	NA

NA: Not Available

A total of one hundred samples from various organs of 16 gaurs were collected for laboratory testing. We could not collect sample from two carcasses because only bone was remained in one carcass (No.12) and another (No.18) was buried before visiting of

investigators. Culture technique failed to identify the pathogenic agents, but PCR technique showed that 50% (8/16) of carcasses were positive to *Clostridium novyi* type B, 6.25% (1/16) to Foot and mouth disease and 12.5% (2/16) to *Anaplasma marginalae*. For toxic substance and heavy metal contamination, the test results were negative to carbamate, organophosphate, organochlorine, warfarin, coumatetralyl, arsenic, zinc phosphide, cyanide, cobalt, chromium, manganese, molybdenum, lead, and cadmium. Nitrate was found at 137 - 2,000 ppm from all ruminal contents (100%). Detail of laboratory test was shown in table 2.

Table 2. Laboratory result of gaur carcasses.

No.	No. of tissue sample	No. of ruminal content sample	Tissue samples			Ruminal content
			<i>C. novyi</i>	<i>A. marginalae</i>	FMD	Nitrate** (ppm)
1	5	1	+	-	-	2,000
2	5	1	-	+	-	778
3	4	1	+	-	-	373
4	7	1	+	-	-	355
5	4	1	+	-	-	371
6	2	1	-	-	-	146
7	11	1	-	-	-	441
8	5	1	+	-	-	648
9	9	1	-	-	-	282
10	5	1	-	-	-	429
11	5	1	+	-	-	588
12	NA	NA	NA	NA	-	NA
13	5	1	-	-	-	2,021
14	4	1	-	-	-	407
15	9	1	-	+	+	137
16	10	1	+	-	-	640
17	10	1	+	-	-	165
18	NA	NA	NA	NA	-	NA

Note: Shown only significant laboratory results,

** Lethal dose in roughage = 4,000 ppm [10]

NA: Not Available

Discussion

The death of 18 wild gaurs (72%) is the country's worst-ever mass death of the giant wild cattle. Before the death, 25 of them regularly foraged for food in the area Kunshorn forest plantation project, Kui buri National Park. The carcasses dispersed in 13 square-kilometre area in the dense forest with high distribution on sex and age of animals (12 male and 6 female, 3 months to 20 years old). They appeared not to have wounds, other metal or bullets and deaths are considered abnormal. Infectious diseases or food poisoning could be hypothesized as the causes of death.

The laboratory results showed *C. novyi* type B 50% (8/16), Foot and mouth disease 6.25% (1/16), *A. marginalae* 12.5% (2/16) and nitrate (100%), and the character of each had been described. *C. novyi* type B is toxin production bacteria being found in soil, intestine and feces. It caused infection necrotic hepatitis in sheep and cattle [7]. Pathogenesis will occur when its spore follows fluke migration to liver. Foot and mouth disease was reported an outbreak in *Bos gaurus frontalis* in Calcutta zoo [4]. The infected animals presented severe mouth and mild foot lesion, and post - mortem lesions revealed massive myocardial damage. While no observable lesion was identified in all complete carcasses, and most of the carcasses (16 carcasses) had full ruminal content. *A. marginalae* is the most common tick born pathogen worldwide and it caused anaplasmosis in cattle. Infection of *A. marginalae* resulted in anemia and death in severe host, while persistent infection developed in some cases [8]. For feed and water related toxicants, especially nitrate toxication, normally plants take up nitrogen from the soil in the form of nitrate, regardless of the form of nitrogen fertilizer applied. The main hazard to ruminants is ingestion of plants that have accumulated excessive amounts of nitrates or nitrites. Death loss from nitrate is an occasional problem in cattle consuming certain annual forages contain over 5,000 ppm nitrate on a dry matter basis [9]. However, the occurrence of nitrate poisoning is unpredictable because toxicity varies among livestock due to age, health status and diet [10]. Moreover, urea and non protein nitrogen toxicant were considered while ammonia smell in various degrees was observed during autopsy. However, only some carcasses (10 of 16) were noted this appearance.

Many infectious diseases which fatal to domestic livestock had considered to be cause of death of wild gaur because there are members of same *Bos* genus. In this case, bacterial culture, viral isolation and the high throughput molecular technique such as RT-PCR and PCR

had applied to verify the causative agents. However, the rotten carcasses with heavy contamination and scatter over with lime (Figure 2, 3) were poor quality samples to hamper the attempt. All carcasses were buried with lime and the biosecurity system had recommended to the entrance, while urea fertilizer was prohibited to prevent nitrate toxication.

Base on the laboratory results, we may not be able to finalize the causative agent of the death. Health condition of live animals in Kunshorn Forest Plantation and nearby domestic livestock should be monitored.



Figure 2. The rotten with heavy contamination of wild guar carcass which remained in the kunshorn forest plantation, Kui Buri National Park, Thailand.



Figure 3. The rotten scatter over with lime of wild guar carcass which remained in the kunshorn forest plantation, Kui Buri National Park, Thailand.

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Reference

1. Groves C. Taxonomy of ungulates of the Indian Subcontinent. *J. Bombay Nat. Hist. Soc.* 2003; 100: 341-361.
2. Prayurasiddhi T, Smith JLK. Field Report: Gaur/Banteng Project April-August 1993. Conservation Biology Program, University of Minnesota, Minneapolis, USA.
3. Duckworth JW, Steinmetz R, Timmins RJ, Pattanavibool A, et al. *Bos gaurus*. 2008. In: IUCN 2013. IUCN Red List of Threatened Species. Version 2013.2. <www.iucnredlist.org>. Downloaded on 28 February 2014.
4. Choudhury B, Mazumder R, Bhattacharya AK. Foot and mouth disease in gayals (*Bos gaurus frontalis*) in Calcutta Zoo. *Rev. sci. tech. Off. int. Epiz.* 1992; 11:797-8.
5. Minett FC. Dissemination of animal disease in India, Role of man and Carrion feeders. *Br. Vet. J.* 1954; 110-9.
6. Glasuddin M, Alam J, Mrahman M. Incidence and distribution of diseases of gayal (*Rosfrontalis*) under semi-intensive management. *Indian J. of Ani. Sci.* 2006; 76(10): 799-801.
7. McVey DS, Kennedy MM, Chengappa M. Virulent of *Clostridium novyi*. *Veterinary Microbiology* 3rd edition 2013. Available on :<http://books.google.la/books>
8. Kocan KM, De La Fuente J, Blouin EF, Garcia-Garcia JC. *Anaplasma marginale* (Rickettsiales: Anaplasmataceae) : recent advances in defining host-pathogen adaptations of a tick-borne rickettsia. *Parasitology.* 2004;129; S285-S300.
9. Whittier JC, 2014. Nitrate Poisoning. <http://www.ext.colostate.edu/pubs/livestk/01610.html>

10. Dan U, Combs D, Shaver R, Thomas D. Nitrate poisoning in cattle, sheep and goats. 1985.
Available on :<http://www.uwex.edu/ces/forage/pubs/nitrate.htm>