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# INVESTIGATION OF THE DEATH OF JAVAN RHINOCEROS (*Rhinoceros sondaicus*) IN UJUNG KULON NATIONAL PARK

Adhi Rachmat Sudrajat Hariyadi[1], Handayani[2], Agus Priyambudi[3], Ridwan Setiawan[4]

[1,2,4] WWF Indonesia – Rhino conservation Program
[3] Ujung Kulon National Park Authority

## Introduction

Javan rhinoceros (*Rhinoceros sondaicus*) is a rare and endangered species that inhabit Ujung Kulon National Park in Banten, Indonesia. This Park holds the only viable population with the population size that is no larger than 50 animals, and the growth rate is estimated at only 1% per year (Hariyadi *et al* 2011). With such a small population showing a slow growth rate, the javan rhino population is vulnerable to pressures such as disease and habitat changes. Emerging disease threats to animal species in the wild consist of: blood diseases (infectious) and breeding diseases (Jonyo 2005). Infectious agents are often found in insect vectors, and toxin from *Lantana camara* plants is known as non-infectious agent that can cause liver damage, as well as mortality in herbivores. In 1982, five javan rhinos were found dead in Ujung Kulon National Park without any conclusive evidence of the etiology of the deaths (WWF-IUCN 1982). In 2010 three rhinos were found dead in three different sites in the peninsula of Ujung Kulon National Park. These deaths are very significant, as they represent 4% of the entire population.

## Materials and Methods

Assessment was done during an annual rhino monitoring trip in May 2010, one team that was assigned to a northern block in the peninsula of Ujung Kulon National Park, came across a pile of skeleton that was identified as skeleton of a javan rhino. Further observations revealed that it was a male rhinoceros based on the horn that was found in the vicinity of the skeleton (within 15 m radius). Approximately 90% of the bones were recovered, and evacuated to the nearest Park post in Taman Jaya village. The position of and the on-site examination of the rhino remains is presented in figure 1. All rhinos were decayed by the time of discovery, and only one carcass showed a complete set of skeletons, and some remnants of the skin, as well as the horn. Therefore, pathological observations were done on site and were based on these materials. Locations of the findings are described in figure 2. Age determinations were done based on the observation of the size of the animal (head to tail length), teeth condition, and horn size. Time of death was judged from the condition of the carcass, and the cause of death was estimated by observing the position of death, presence of bones and other tissues in the death site, as well as analyzing the presence of infectious agents from soil, water, and insects (*Tabanus malayensis*, and ticks).

Soil sample was taken from the locations using scoop to dig approximately 10 cm from the surface, and the water sample was taken from rivers and creeks within 1 km radius from the death sites. Soil and water samples were taken to SUCOFINDO lab in Cilegon Banten for bacteria identification, while the insect samples were analyzed using DNA identification in Molecular biology lab in IPB (Bogor Agriculture University).

### Sample collection

The tissue samples of insect were collected from an area within 1 km radius from the rhino death sites in Ujung Kulon National Park. Insect samples were fixed in ethanol (90%) and were stored at -20$^{o}$ C for molecular studies.

Sample preparation (DNA extraction)

DNA was extracted using a slightly modified protocol developed for DNA extraction. The samples were taken from reaction tube into eppendorf tube 1.5 ml, washing by Tris-EDTA with low concentration (*low* TE), add *lysis buffer.* The next steps are removing supernatant, add washing and digestion buffer into the sediment and incubated in incubator on the temperature $55^0$C for one night. After that, giving phenol into samples as much as 500 µl and adding 500 µl the chloroform isoamil alcohol. The surface of its solution moved into new tubes, add absolute ethanol 2x volume. The DNA sediment was washed by ethanol 70%. DNA result dried in room temperature. DNA dissolves in TE solution then incubated in water heater in $37^0$C for 15 minutes. Subsequently DNA samples save in temperature $20^0$C. DNA quality detected by migrated in agarose gel 1.2% and seeing by UV light.

DNA amplification

Polymerase chain reaction was carried out in thermo cycle apparatus (Perkin-Elmer 480 and MJ thermal cycle) using primers for trypanosome (generic, *T evansi*, and *T brucei* coded as: TR3 and TR4. The PCR in condition: pre-denaturation in temperature $94^0$C, denaturation in $94^0$C for 45 seconds, primary sticking stage (*annealing*) is in $51^0$C, *extension* is in 72 $^0$C with repetition 35 cycles. PCR reaction is ended by polimerization (*final extension*) in 72 $^0$C.

Another PCR reaction of each mixture is 50 µl with water in composition 34.25µl, 5µl buffer PCR, MgCl 2.5 µl, 1 µl dNTP mix, using primers; TR3 and TR4(Chokesajjawatee N, Panyim S, 1993) , 5 µl DNA templete dan 0.25 µl Taq DNA polymerase (Promega).The PCR amplification was performed for 35 cycles. The PCR products were analyzed by agarose gel electrophoresis at 80 volts. Gels were stained with ethidium bromide and the DNA fragment was visualized under UV light.

**Results**

The rhino was found as skeletons with maggots feeding on the horns and the nails of the foot. Body parts other than the bones, horns and nails were completely decomposed. Bones were stained with red patches (fungus), and the pelvic girdle was broken in exactly two halves. The teeth, especially the molars, were sharp with only slight indication of wears. There were 6 molars instead of recorded 7 molars. Total length of the rhino was estimated at 270 cm with addition tail length of 55 cm. Horns, the lower small incisors were found on location. Missing bones included: some digits, sternum, a small incisor, and the tip of tailbone. The rhino seemed to have leaned to the right side as indicated by the position of right ribs that were superimposed by the vertebral column, and the left ribs. The skeleton was oriented north-south with the head in the north direction (bearing 3400). The nails of fore and hind legs were proportionally situated in the west side of the ribs/vertebrae pile. The nails of the right hind leg were embedded 5-7 cm in the soil (deeper that the other three nails).

Current Findings

Skeletal length and dentitions suggested that the animal was a young adult, and possibly male. It also showed that the position of death was leaning to the right side, suggesting that the death was not due to old age (compared to the death in 2000, death of old age was related to a hidden location and a prone position).

Based on the condition of the carcass, the rhino's time of death was estimated at 2-3 months ago, and poaching can be ruled out, as most targeted parts (horns and incisors) were found intact on location. There were signs of scavenger animals feeding on the rhino tissues and dragging the head apart from the rest of the pile.

Judging from the horn size and dentition, it is agreed that it was an adult rhino, but the dentition suggested that the rhino was not at a very old age, as most of the crowns of the teeth (molar) was still in good condition (sharp). Based on the conditions of the surroundings, no signs of struggle were found, and no signs of extreme pain were observed.

Therefore, it can be assumed that the rhino died very quick (almost a sudden death). Documentation of rhino deaths in the past showed that sudden death could be caused by: carditis, trypanosome, and virus (Infectious bovine rhinotracheitis-IBR). However, previous investigations of diseases among water buffaloes in the villages showed prevalence of SE and blood parasites (theleria).

Soil analysis is negative for anthrax, but positive for *Clostridium perfingens*. This bacterium is a common following a decaying/decomposing carcass, although contamination of such species can cause illness in some animal species. Water and soil sample are also negative for other bacterial contaminants (*E.coli* and *Salmonella spp*). The soil and water samples were negative for mercury. The investigation was continued by surveying the possible vectors for Trypanosomes, a common cause of mortalities in African (Clausen 1981) and Asian wildlife.

Result of DNA identification of *Trypanosoma spp*

PCR Analysis on Tabanid flies (*Tabanus malayanensis*) and ticks collected from the death site using generic *trypanosome* marker confirmed the presence of this parasite in 5 of 6 tabanid flies' samples. Further PCR analysis using marker for *T. Evansi* resulted in the identification and amplification of DNA fragments (257 base pairs in four samples and 350 base pairs in one sample) belonging specifically to *Trypanosoma evansi*; Thus confirming the presence of *T. Evansi* in the Tabanid flies.

**Discussions**

It can be concluded that there are risk of infectious diseases might be the cause of mortality of Javan rhinoceros, as helminth parasites have been previously detected (Tiuria *et a*l. 2006). The highest risk may come from blood parasite *Trypanosoma evansi*. It is recommended that the population manager pay attention to the possibility of interaction between livestock in the surrounding (consisted mainly of water buffalo) with Banteng (*Bos javanicus*), and javan rhinos; thus providing necessary measures to prevent disease transmission. In addition to that, the quality of rhino habitat needs to be improved to facilitate the growth of food plants containing high nutritional values to help rhinos cope with the disease risk, as well as the changing habitat.

**Acknowledgment**

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A

B

**Figure 1**. Position of the skeletal remains in the forest of Ujung Kulon National Park (A) and the on-site examination of the skeleton (B).

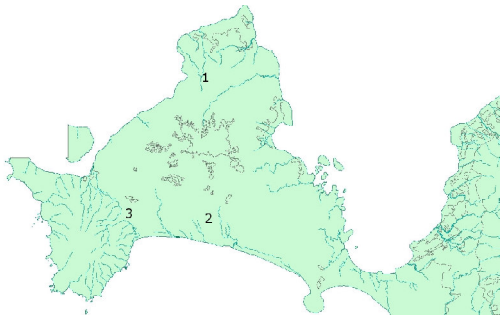


**Figure 2**. Locations of the findings within Ujung Kulon national Park marked as 1,2, and 3. The complete set of skeleton was found in location number 1.