

Bone Morphometric Analysis Study to Advance Javan Rhinoceros (*Rh. sondaicus*) Post-Mortem Demographics and Resultant Species Public Health Impact

Master of Public Health Thesis

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The rise of emerging diseases, with an estimated 75% of future diseases anticipated to be zoonotic in origin, is a major concern for global public health. In addition to the concern of wildlife conservation and preservation of biodiversity, protection of the Javan rhinoceros is essential in terms of cultural significance, ecological integrity, and political security with regard to the illegal trafficking associated with rhinoceros horn. An unprecedented epidemic of Javan rhinoceros death was reported in the last protected area of the species, Ujung Kulon National Park. With disease such as anthrax present in the soil and trypanosomiasis decimating the population of their relative, the Sumatran rhinoceros (*Rh. sumatrensis*), in nearby Malaysia, characterization of this disease is vital in order to determine its impact on the remaining population and the ecological effects on other species, including domesticated agricultural animals of nearby populations and that of people.

The rarest of all living rhinoceroses is the Javan rhinoceros (*Rhinoceros sondaicus*) found today in only one location –Ujung Kulon National Park (UKNP) in West Java, Indonesia. Though once prolific throughout Asia, the last two subspecies of Javan rhinoceros (*Rh. sondaicus floweri* and *Rh. sondaicus inermis*) went extinct in Nepal, India, Burma, Peninsular Malaysia, and Sumatra by the 1930's and as recently as 2010 in Vietnam (Brooke *et al*, 2011). The primary cause of this decline is attributed to the poaching of Javan rhinoceroses for their horn and other body parts for Traditional Chinese Medicine practices, supporting an unfounded belief that rhinoceros horn possesses “magical” properties to cure fever, arthritis, high blood pressure, and even cancer (Foose and van Strien 1997).

During the last International Conference for the Javan Rhinoceros summit in Indonesia in 2014, a priority to establish a second population of Javan rhinoceros was announced with Cikepuh Wildlife Reserve proposed to be the host. Not only is the current population of no more than 60 animals at risk of extinction from poaching, natural disaster (i.e. tsunami or volcanic eruption), and disease (i.e. trypanosomiasis), but also they also are thought to have reached the carrying capacity of their current ecosystem (Hariyadi *et al*, 2011). However, prior to translocating animals to regions that may increase chance to human exposure, a full comprehension of their epidemic needed to be characterized.

Several epidemics within Ujung Kulon National Park resulted in acute rhinoceros death of 8.9% of the population commencing first in 1982. The rhinoceros deaths did not appear attributable to poaching due to the finding of rhinoceros horn near or on five decayed bodies and the assumption that poaching is often initiated to gain the highly sought after horns for the black market. Therefore, differentials for the cause in increased mortality rate include death from environmental factors such as toxins, disease, inter-species aggression, and other demographic-

related conditions (i.e. natural geriatric death, parturition). The most likely hypothesis for acute mortality rests in the endemic presence of low seropositivity (1.8%) of *Pasturella multocida*, the causative agent of Hemorrhagic septicemia, in local captive water buffalo (Hariyadi *et al*, 2011). These carrier animals may be contributing to outbreaks within the national park. Additionally, anthrax outbreaks have also been documented in the region.

Proposed signs of progressive disease culminating in death of some deceased individuals included ambulation with feeding, diarrhea, resting recumbency, spasmodic leg movements, and climaxed by mortality. One fresh carcass was found to have rectal prolapse and mucinous ptyalism from both the mouth and nostrils.

Other differentials or risk factors would gain more weighted significance pending additional population demographic information, such as if deaths due to a geriatric condition or if they were due to pregnancy-related complications.

Because the majority of these animals are found decomposed post-mortem, a new field-based methodology of determining the age and sex of Javan rhinoceros based on their orthopaedic structures is needed. To devise a method to characterize decomposed rhinoceros post-mortem required studying all available samples, including controls and cases (unknown and known sex and/or age specimens). Once a basis for characterizing demographics can be established, the methodology will be applied to the population of recently deceased rhinoceros from Ujung Kulon National Park and then even to future fatalities. To this end, determining which individuals are more susceptible to the underlying cause of outbreaks can be ascertained from this study.

Although an argument can be made for protection of this critically endangered species due to the importance of maintaining biodiversity and hotspots, additional models and studies proving benefits of biodiversity are still needed. Varying computational algorithms yield different interpretations of the pros and cons to biodiversity due to difficult-to-model confounding variables. However, three central public health concerns related to Javan rhinoceros health advocate for their conservation: 1) human and livestock health in control of infectious diseases; 2) serving as sentinels for environmental health; 3) cultural symbolism.

A. Infectious Disease

As previously mentioned, the suspected greatest threat to their immediate conservation, other than human-related land-encroachment, habitat infringement, and poaching, is susceptibility to infectious disease. Hemorrhagic septicemia is a fatal disease of ungulates, particularly cattle, yak, camel, and water buffalo. *P. multocida* can remain in the tonsils of healthy buffalo and be shed during times of stress, such as high temperature and humidity, concurrent infection, poor nutrition, or higher work demands; outbreaks more commonly occur during the rainy season where there are not only increased stressors but also improved moist conditions for pathogen survival time. Infection is transmitted via oro-nasal secretions (carrier or infected animals) or through contaminated feed/water. Water buffalo surveyed in nearby farms by Ujung Kulon National Park that had positive seroprevalence were associated with lack of a permanent night

housing area, low body condition score, hyperthermia, historical acute death or clinical signs within the year, and permitted grazing within the park. It is suspected that carrier water buffalo are responsible for transmission of the disease both within these farms and potentially to the dead-end host rhinoceros. In 1981 an outbreak resulting in the death of 350 domestic goats and 50 buffaloes took place near the park. Following the 1982 rhinoceros outbreak, a local government vaccination program was implemented, though continual preventative implementation has been inconsistent (Khairani *et al*, 2019).

Although the causative *P. multocida* serotypes of Hemorrhagic septicemia do not cause human infections, many serotypes have the potential to infect people. Therefore, preventative practices (i.e. vaccination, improved husbandry, and serologic testing) of domesticated livestock is essential to prevent disease outbreaks in other animals and prevent potential zoonotic disease. Additionally, until further data confirms the seroprevalence of *P. multocida* in Javan rhinoceros, prevention of wildlife contact should be recommended (The Merck Veterinary Manual).

Bacillus anthracis, the spore-forming causative bacterium of Anthrax, is common in wild and domestic herbivores and seen in people exposed to contaminated animal products/tissues or direct inhalation of *B. anthracis* spores. Clinical signs differ based on route of infection, host factors, and strain-specific factors. It is commonly associated with acute septicemia and high fatality. Spores remain viable, though dormant, and represent a direct risk of infection for grazing livestock. Additionally, biting flies may mechanically transmit *B. anthracis* from one host to another.

Like in this geographic location, globally the prevalence and true incidence of anthrax remains unknown. Epizootics tend to be associated with drought, flooding, soil disturbance, and large gaps in time between outbreaks. In developing nations, it is suspected that for each affected cow, up to 10 human cases can result (The Merck Veterinary Manual).

Although there is no data regarding Javan rhinoceros susceptibility to anthrax, it remains possible that they can serve as sentinels or potentially even carriers of disease. Regardless, preventative measures (i.e. vaccination, clean husbandry methods, and control of scavengers feeding on deceased infected animals) should be taken in endemic regions to protect livestock and potentially even higher-risk associated individuals.

Trypanosomiasis is caused by protozoa from the genus *Trypanosoma* and causes disease in all domestic animals, with two known species resulting in zoonotic disease. The water buffalo are a known disease reservoir and suspected of being the reservoir of disease for the outbreak of *Trypanosoma evansi* that killed naïve, captive Sumatran rhinoceros in Malaysia. Trypanosomes are transferred from biting flies, and specifically, the Tabanid fly in the case of Surra in Sumatran rhinos and near Ujung Kulon National Park. It is the suspected culprit for transmission of Surra in rhinoceros. Surveillance for *T. evansi* in 2014 revealed 90% prevalence of trypanosomiasis in livestock of two villages in direct contact with the national park. Concerns regarding Surra aside from mortality and morbidity to livestock, include economic loss from decreased productivity,

reduced weight gain, decreased milk yield, reproductive losses, and financial burden for treatment (Iowa State University, 2015).

In summary, for all these diseases, there remains an unknown attack rate in Javan rhinoceros, domesticated livestock, or local people. For zoonotic transmission, the spillage into the human population remains unknown due to poor documentation or absence of current disease that remains a potential emerging threat. Identifying risk factors of susceptible populations (rhinoceros, people, and livestock) and ecosystem balances (i.e. tabanid vector biology and *B. anthracis* soil prevalence) are all essential for preventative measures of life-threatening diseases.

B. Environmental Impact

The Javan rhinoceros is a keystone species and thus plays an integral role in restoring degradative ecological processes. Their vital role in tilling soil, degrading older vegetation for new growth formation, and spreading seeds through their faecal material is suspected to be important in maintaining resource distribution based on analogous species in other geographic locations. Data to confirm this is lacking. By establishing a second viable population, we hope to preserve the environment through the rhinoceros' ecosystem services and simultaneously maintain biodiversity. Few ecological studies have been done on Asian species of rhinoceros. Overall, a lack of data regarding megaherbivores localized to a dense tropical forest habitat remains. Similarities regarding their impact have been deduced from the more widely studied and closest related megaherbivore, the white rhinoceros (*Ceratotherium simum*), and weak comparisons from *Loxodonta africana*, *Loxodonta cyclotis*, and *Elephas maximus*. One key difference, though, is that *C. simum* is a megagrazer.

However, the white rhinoceros as a comparative species has had more comparable research. A thirty-year aerial survey of reintroduced white rhino into Kruger National Park showed that areas dense with rhinoceros had twenty times more grazing lawns, which are regions of specific and varied grass growth that species other than rhinoceros such as zebra, gazelle, warthog, and antelope utilize, than did areas with fewer rhinoceros. In fact, there was 60-80% less "short grass cover", a general metric that describes plant diversity in grassy African lands (Cromsigt et al, 2014). This increased heterogeneity of grass and increase in short grass cover is suspected to have cascading effects, such as creating habitat for certain species of birds (Krook, Bond & Hockey, 2007), grasshoppers (Joern, 2005), small mammals (Engle et al, 2008), and ungulates (Verweij et al, 2006), changing fire regimes (Owen-Smith, 1988), and tree-grass dynamics (Bond, 2008). Soil health, as exhibited by its turnover of diverse and productive species, helps prevent soil erosion and water health. Impala and warthog feedings are suspected to be facilitated by white rhino, as they feed on the grazing lawns initially created by white rhino (Cromsigt & Olff, 2008; Waldram, Bond & Stock, 2008). Overall, this impacts human health in terms of crop productivity, water quality, relations with the biodiverse animal species, and reduction of human-animal contact if plentiful grass reduces the preponderance of competition between domestic species with wild ones. Analogously, one may draw a similar conclusion to the Javan rhinoceros and its correlated environmental impact. Ensuring the success of the Javan

Rhinoceros, a vital rainforest species and the most predominant mega-herbivore of the region, may prove essential to this homeostasis.

Due to their large body mass, characterized as equal or greater than 1,000 kg, megaherbivores are limited more by food availability than top-down predation and thus play an impactful role on their ecosystems (Owen-Smith, 1988). This led to the hypothesis that Pleistocene extinctions of megaherbivores such as mastodons and mammoths changed the ecosystem in drastic ways such as modifying fire regimes, floral ecology (Gill *et al*, 2009) and thus instigating extinctions of affected taxa. This has led to the contemporary idea known as the “rewilding philosophy” whereby re-introduction of extant megaherbivores into these ancient, degraded ecosystems may result in its restoration (Donlan *et al*, 2006). Studies of extinct grazing megafauna review their impact mainly on impacting biome shifts (Zimov *et al*, 1995), global warming (Doughty *et al*, 2010), decreased availability and movement of nutrients (Doughty *et al*, 2016), vegetation changes (Gill, 2014), reduction in carbon sequestration (Doughty *et al*, 2016), and alterations in global methane (Smith, Elliott, & Lyons, 2010; Hempson *et al*, 2017).

Less than 10% of pre-Pleistocene extinction nutrient transport capacity has been retained in today's biomes (Doughty *et al*, 2013). This has been attributed to a decrease in species and individual numbers of large mammals that move nutrients laterally. Megaherbivores directly impact vegetation through not only selective foraging (Plas *et al*, 2016) and seed dispersal but also physical impacts (i.e. behavioural reorganization of fauna architecture) (Landman *et al*, 2014). Although there is crossover of species contributing to the biodiversity of vegetation, they collectively maximise their efforts through functional complementarity. For example, Asian tapirs have been shown to be unable at dispersing the largest seeds, which Asian elephant are able to do (Campos-Arceiz A *et al*, 2012). The effects of megaherbivores on tropical rainforests remain relatively unknown. It was proposed that many Neotropical trees have “megafaunal” fruits due to being dispersed by megafrugivores, which has since decreased since the Pleistocene gomphothere extinction. Additionally that reduced tree recruitment and the geographical ranges of a variety of tree species, such as hardwoods that have higher wood density, have been modeled to have high carbon storage, and their loss will proportionally decrease the lead to a loss of the Neotropical carbon sink (Blaket *et al*, 2009; Janzen & Martin, 1982; Bello *et al*, 2015; Doughty *et al*, 2016).

Carbon losses as vast at 2-12% in most international tropical forests and increased carbon storage are expected to occur if there is a loss of the last megafrugivores (Africa and Asian forest elephants) and American tapirs. However, no studies have included rhinoceros (Osuri AM *et al*. 2016, Sobral *et al*, 2017; Bello *et al*, 2015; Poulsen JR *et al.*, 2017).

Lastly, methane, a known contributor to trapping of radiation (radiative forcing) resulting in elevated global temperatures, is a known byproduct of anaerobic microbial fermentation of plant materials of the rumen, colon, or caecum of herbivores. Ruminants produce more methane than hindgut fermenters such as equids or rhinoceroses, or non-ruminant foregut fermenters, such as macropods and sloths. More methane is produced as ruminant mass increases, while this is an inversely proportionate relationship of hindgut and non-ruminant foregut fermenters. It is

suspected that one contribution to increased global methane production is secondary to a replacement of megaherbivores, which are predominantly hindgut fermenters, with an increase in ruminant livestock. This replacement in Africa increased emissions from 3.4 to 8.9 Tg yr-1 (Hempson *et al*, 2017). Although the ramifications of climate change on global public health are known, the precise repercussions for replacement of domesticated ungulates with hindgut megaherbivores remains unknown, and depending on the location in the world, no suitable megaherbivores could be practically reinstated. However, it is suspected that there would be a decrease in greenhouse gas emission, thus reducing radiative forcing, and thus reducing global warming.

C. Cultural

In addition to its ecological benefits, the Javan rhinoceros serves as a prominent cultural symbol throughout Javan and Cambodian culture both historically to the present. They have been depicted in the temple of Angkor Wat in Cambodia built between 1040–1120 AD. The rhinos in these depictions are shown carrying the fire god, Agni and in another relief are condemning the “damned” in a heaven/hell. They are also depicted in the temple of Ta Prohm. Although taught in school of their rhinoceros and the presence of local pride, locals have minimal association with the species due to less media and awareness coverage for the rhinoceros. Larutan Penyegar, an Indonesian soft drink company, displays their version of the Javan rhinoceros, but their illustrator portrayed the two-horned white rhinoceros in its stead due to lack of awareness and imagery allotted to their own national rhinoceros.

In summary, it is vital to appreciate the increasing interactions between humans and wildlife due to encroachment of untouched, “virgin” habitats from increasing population sizes and proportional agricultural needs. A sudden spike in zoonotic diseases have been encountered over the past quarter century from diseases such as Ebola, Hendra, SARS, and H5N1. Specifically, at least 61% of all human pathogens are zoonotic and represent 75% of all emerging pathogens this decade alone. Zoonoses not only impact human health but can also devastate livestock production. Like many zoonoses, such as Ebola whereby human mortality rate spiked in addition to noting a parallel in association with 25% of 43 wild chimpanzees were found dead in the Tai National Park, Cote d’Ivoire. It has been reported by the Jane Goodall Institute that since the first epidemic in 1976, Ebola may have killed a third of the world’s chimpanzees and gorillas. This disease has since held enormous consequences for affected humans.

Jakarta, Indonesia is the most populous city in the Republic of Indonesia with over 30 million people inhabiting the city. Habitat encroachment of rural Java has been increasing, and with a population with such a large population and high density (14,464 people/square kilometer), any epidemic is of concern. The presence of anthrax in the Indonesian soil is commonplace knowledge. However, the sudden epidemic of the most critically endangered species of rhinoceros stirs concern, as the cause still remains unknown, though hemorrhagic septicemia is the most likely etiology. Additionally, summarized are the potential ecological impact of this keystone species and the paucity of data regarding its previous and current environmental role.

The findings of the study are attached for further review with hopeful submission for journal publication in the near future. This study quantifies, for the first time, age and sexual dimorphism of the species based on calvaria and dental patterns from skeletal remains with potential application to living and post-mortem specimens. Doing so will enable in-field conservationists to determine demographics, namely age structure and gender of individuals, from the recent epidemic of Javan rhinoceros deaths found in Ujung Kulon National Park. These demographics are needed to better characterize risk-associated mortality and aid in differentials to the cause of mortality. Additionally, this data can aid in understanding if and how the epidemic can affect human health, in consideration how human-animal conflict resulted in the recent extinction of the last population of Sumatran rhinoceros from trypanosomiasis carried by water buffalo harvested by nearby farmers.

Therefore, my role as a veterinarian with a background in orthopaedic medicine was needed in order to perform this study. Additionally, in an answer to the conservation crisis of this species, I also was involved at the International Conference for the Javan Rhinoceros where invested parties collaborated on a plan to save this IUCN red listed species from extinction. My role was to offer veterinary advice, such as appropriate anaesthetic techniques and transportation methodologies, in order to help determine how much space each new translocated rhino would need in order to mate and be successful as a population.

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The methodology is elaborated in "methods" section. It mainly consisted of assessing skull measurements of all available specimens of Javan rhinoceros in the USA, UK, Paris, and Indonesia, studying dental architecture, and surveying rangers to accurately determine gender of specimens that had not decayed post-mortem (i.e. identifiable presence of a phallus for a male).

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