

THE STATUS OF *Oryctes rhinoceros* Nudivirus (OrNV) INFECTION IN *Oryctes rhinoceros* (Coleoptera: Scarabaeidae) IN INDONESIA

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ABSTRACT

Oryctes rhinoceros is a major problem on oil palm in Indonesia, especially during replanting. *Oryctes rhinoceros* Nudivirus (OrNV) is a virus that infects both larvae and adults of *O. rhinoceros*. An extensive survey of OrNV infection on *O. rhinoceros* in Indonesia has not been conducted. The objective of the research is to identify the rate of OrNV infection in its host from various sampling sites in Indonesia. Adults and larvae of *O. rhinoceros* were collected from Sumatra, Belitung, Java, Kalimantan and Sulawesi. Infected larvae were determined by their physical character, i.e. prolapsed rectum, while infected adults were determined by dissection to observe the swollen midguts. The incidence of OrNV infection in larvae was difficult to estimate, as only 11 out of 417 larvae showed prominent symptoms. OrNV infection rates in adults *O. rhinoceros* in oil palm plantations in Sumatra, Belitung and Kalimantan were between 64% and 90%, and female *O. rhinoceros* could still produce eggs even when they were infected by OrNV. In Sulawesi and Java, which are not major oil palm plantation centres, OrNV infection rates were below 16%. It is suspected that most of the *O. rhinoceros* population from areas intensively cultivated with oil palm is persistently or latently infected by OrNV and the beetles remain fertile.

Keywords: biological control agents, infection rates, Nudivirus, survey.

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INTRODUCTION

The current status of *Oryctes rhinoceros* in Indonesia is that it is the main pest in coconut and oil palm plantations (Susanto *et al.*, 2012) and harmful outbreaks of *O. rhinoceros* frequently developed in replanted areas (Zelazny *et al.*, 1992; Chenon

and Pasaribu, 2005; Salim and Hosang, 2013). Decomposed coconut and oil palm trunks provide extensive *O. rhinoceros* breeding sites, and zero burning regulations have been contributing to rising *O. rhinoceros* populations (Purba and Sudharto, 2000; Abidin *et al.*, 2014). Extremely large numbers of *O. rhinoceros* have been found in oil palm plantations, with a 2 x 2 x 0.2 m³ sample of decomposed empty fruit bunches containing 4441 larvae, 12 pupae and 201 adults having been reported (Purba *et al.*, 1999). Larval populations in natural coconut breeding sites were smaller than in oil palms, at only 3 - 48 individual for 1 x 1 x 0.5 m³ (Indriyanti *et al.*, 2017).

The discovery of the pheromone active ingredient, ethyl 4-methyl octanoate, as an attractant for *O. rhinoceros* adults revolutionised

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environmental pest control (Renou *et al.*, 1998; Chenon *et al.*, 2001). Chenon and Pasaribu (2005) reported that thousands of adults had been trapped with pail pherotraps in North Sumatra. Combination of several control methods for *O. rhinoceros* have been suggested, such as manual pick-up of larvae from decomposed palm trunks, bio-traps made from decomposed empty fruit bunches, *Metarhizium anisopliae* application in breeding sites, camphor treatment in young shoots, manual pick-up of adults from their tunnels, and pheromone traps (Purba *et al.*, 1999; Susanto *et al.*, 2012). Although various control methods have been used, *O. rhinoceros* remains a substantial problem. Pheromones have been found to be effective but expensive, and only large private oil palm companies are capable of using pheromones for control so far. The cost factor has prevented the use of commercial pheromone products by small farmers.

OrNV is non-occluded Baculovirus belong to family *Nudiviridae*, genus *Alphanudivirus* (Jehle *et al.*, 2013). OrNV infects larval and adult stages of *O. rhinoceros* in midgut epithelial cells and the fat body (Huger, 2005; Jehle *et al.*, 2013). Bedford (2014) states that OrNV has been used as a main control of *O. rhinoceros* in non-endemic areas such as the Pacific; unfortunately, however, in areas such as Indonesia and Malaysia, OrNV has not been a main method of control. Hopes for effective OrNV utilisation lie in the differences of pathogenicity between isolates. There

are some slight enzymatic restrictions of EcoRI and HindII patterns in Indonesian isolates (Kobayashi and Somowiyarjo, 1995). The most virulent OrNV isolate, isolate B, has delivered significant results for *O. rhinoceros* control in Malaysia (Ramle *et al.*, 2005). Benefits of OrNV as an *O. rhinoceros* biological control are that it is environmental-friendly, cheap and permanent.

Survey of OrNV incidence across a wide area in Indonesia have not been conducted. Indonesia is a place of origin of OrNV (Huger, 1966; Bedford, 2014) and it is thought to be found as various isolates with different levels of pathogenicity. This article presents preliminary results of the status of OrNV infection rate in *O. rhinoceros* taken from the survey. Preserved *O. rhinoceros* raw organ containing OrNV will be used for pathogenicity tests.

MATERIALS AND METHODS

Sampling Locations

Third instar larvae and adults of *O. rhinoceros* were collected from Simalungun, North Sumatra; Rokan Hulu, Riau; Sijuk, Belitung; Sukabumi, West Java; Tayu, Central Java; East Kotawaringin, Central Kalimantan; Penajam Paser, East Kalimantan; Mamuju Utara, West Sulawesi; and Morowali, Central Sulawesi (Table 1).

TABLE 1. LOCATION, THE NUMBER OF *Oryctes rhinoceros* (Coleoptera: Scarabaeidae) AND GLOBAL POSITIONING SYSTEM (GPS) COORDINATES OF SAMPLING SITES

No.	Location	GPS coordinates	<i>O. rhinoceros</i>	
			Adults	Larvae
1	Marihat Baris, Siantar sub-district, Simalungun Regency, North Sumatera	2.9223, 99.0894	57	50
2	Kalianta Estate, Rokan Hulu Regency, Riau	0.465870, 100.820694	61	50
3	Sijuk Village, Belitung Island	-2.55847, 107.74773	50	50
4	Parungkuda sub-district, Sukabumi Regency, West Java	-6.844, 106.7526	50	50
5	Bulumanis Lor, Margoyoso sub-district, Pati Regency, Central Java	-6.608368, 111.068438	60	50
6a	Rubung Buyung, Cempaga, East Kotawaringin Regency, Central Kalimantan	-2.19719, 113.06786	74	-
6b	Pundu, Cempaga Hulu, East Kotawaringin Regency, Central Kalimantan	-1.97657, 113.10685	-	50
7	Babulu Darat, Babulu sub-district, Penajam Paser Utara Regency, East Kalimantan	-1.495287, 116.406840	-	50
8a	Kumasari, Sarudu sub-district, Mamuju Utara Regency, West Sulawesi	-1.701959, 119.367979	54	-
8b	Tammarunang, Duripoku sub-district, Mamuju Utara Regency, West Sulawesi	-1.648731, 119.411832	-	50
9	Beteleme village, Lembo sub-district, Morowali Regency, Central Sulawesi	-2.1396, 121.2839	3	17
Total			409	417

Collection of *O. rhinoceros* Adults and Larvae

Adults were trapped by pail pherotraps equipped with vanes. Larvae were collected from decomposed coconut and oil palm trunks, decomposed sawdust and other organic materials. Collected samples were sent to the Insect Pathology Laboratory, Plant Protection Department, Bogor Agricultural University between January and April 2018.

Observation of OrNV Infection in Larvae and Adults by Physical Characters

Larvae were identified using a simplified field key for *O. rhinoceros* (Beaudoin-Ollivier *et al.*, 2000). Infected larvae were identified by swollen, transparent abdomens. Sometimes the abdomen has a white, shiny, pearly appearance (Huger, 1966). Heavily infected larvae also show signs of prolapsed rectum (Huger, 2005). Larvae were observed for the OrNV infection by external characters as described by Huger (1966; 2005) and also on their digestive tracts. The digestive tract was cleaned with sterilised aquadest and the midgut sections were observed.

Adults were measured from horn to abdominal tip. Fresh adults were opened along the line between the dorsal and ventral abdomen. Infected adults were characterised by a whitish swollen gut (Crawford and Zelazny, 1990; Burand, 1998; Huger, 2005) and their eggs were counted.

RESULTS AND DISCUSSION

OrNV Infection Rate in Larvae and Adults

Fifty larvae were obtained from each of the nine sampling points except for Morowali, Central Sulawesi, from which only 17 were collected (Table 1). Prolapsed rectum was found in only three larvae from Riau, while only four larvae with white swollen abdomens were found in each of the Riau and Belitung sites (Figures 1 and 2). Larvae with a prolapsed rectum had more hemolymph liquid but there were no differences between their guts and those of the healthy larvae. Infected larvae can only be identified by their external body symptoms. There were 406 larvae that had no external symptoms, and a molecular method might be more accurate for OrNV detection than by relying on physical observation.



Figure 1. Motionless third instar larva of *O. rhinoceros* with prolapsed rectum as a strong symptom of Nudivirus (OrNV) (Huger, 1966).



Figure 2. Development of Nudivirus (OrNV) infections from mild to severe. Larvae (a) was still alive; larvae (b) and (c) were alive but motionless; and larvae (d) was dead with a prolapsed rectum.

From eight sampling sites, 409 adults were collected: 251 females and 158 males (Table 1). There were 207 adults with white swollen midguts (Figure 6); the rest had transparent, beige, brownish, or black midguts (Table 2; Figures 3, 4 and 5). Those adults with white midguts had gut diameters ranging from 2 to 4 mm. Those with a larger midgut diameter contained a large amount of white liquid and were fragile. Adults with brown, beige, and transparent midguts were assumed to be healthy (Crawford and Zelazny, 1990). Those with black midguts appeared to be ailing and were moving less than healthy specimens.

TABLE 2. NUDIVIRUS (OrNV) INFECTION STATUS IN *O. rhinoceros* ADULTS BASED ON COLOUR OF THE MIDGUT

Midgut colour	Status: healthy	Status: infected	Total
Brown	158	-	158
Beige	24	-	24
Black	9	-	9
Transparent	11	-	11
White	-	207	207
Total	202	207	409

Population of adults from eight sampling sites had different degrees of OrNV infection. Adults

from Riau and North Sumatra had high rates of OrNV infection, at 90.2% and 89.5%, respectively, while adults from Java and Sulawesi had low OrNV infection rates of below 16%. OrNV infection rates in Belitung and Central Kalimantan were also high at above 60% and 70% (Figure 7). It has been suggested that there is an interaction between intensity of oil palm plantation and the incidence of OrNV infection.

Oil palms have been cultivated since 1911 in North Sumatra, 1922 in South Sumatra, and 1981 in Kalimantan (Pamin, 1998; Suprianto *et al.*, 2016). High OrNV incidence was found to be concomitant with intensive oil palm cultivation. In contrast, Java and Sulawesi are not intensively cultivated with oil palms and have low OrNV incidence. Areas under oil palm cultivation are 36 163 ha in West Java and 354 000 ha in Sulawesi (Directorate General of Estate Crop RI, 2016; Gapki, 2015). Bedford (2013) has reported low OrNV incidence of 7%-25% in the Philippines and medium-to-high OrNV incidence of 41%-75% in India. Oil palm is the most important commodity crop in Malaysia with total planted areas of over 4.9 million hectares (MPOB, 2011). OrNV incidence in Malaysia has been reported as high as 75%-100% (Ramle *et al.*, 2005). High OrNV incidence in Malaysia is also concomitant with intensive oil palm cultivation.



Figure 3. Transparent midgut of *O. rhinoceros* adult.

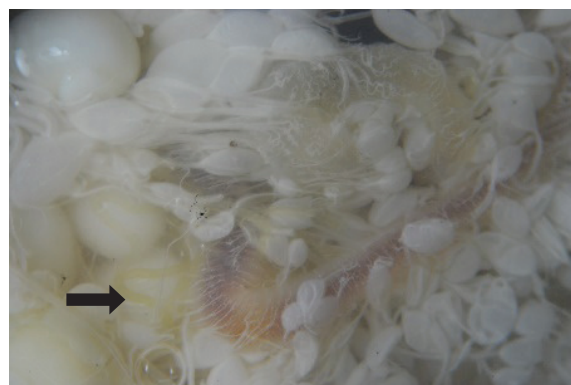


Figure 4. Brown midgut of *O. rhinoceros* adult.

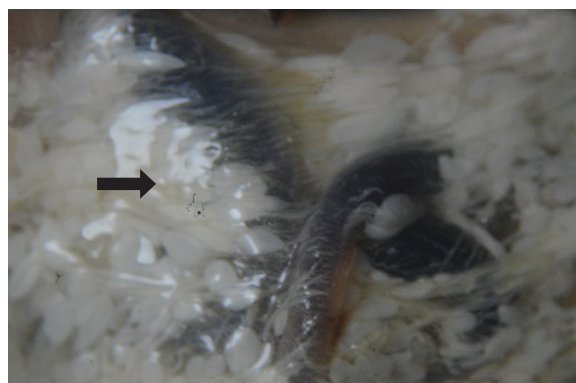


Figure 5. Adult of *O. rhinoceros* with black midgut. This beetle appeared to be ailing and was moving less.

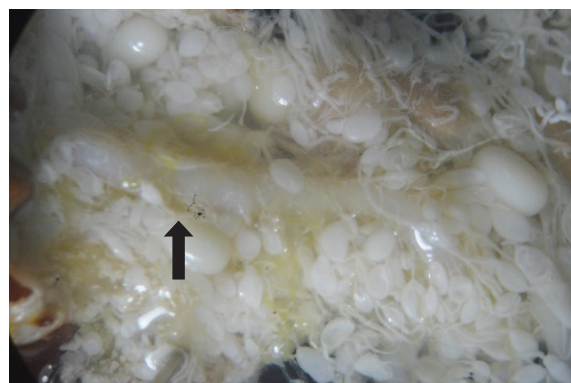


Figure 6. White, swollen midgut as a feature of obvious Nudivirus (OrNV) infection in *O. rhinoceros*.

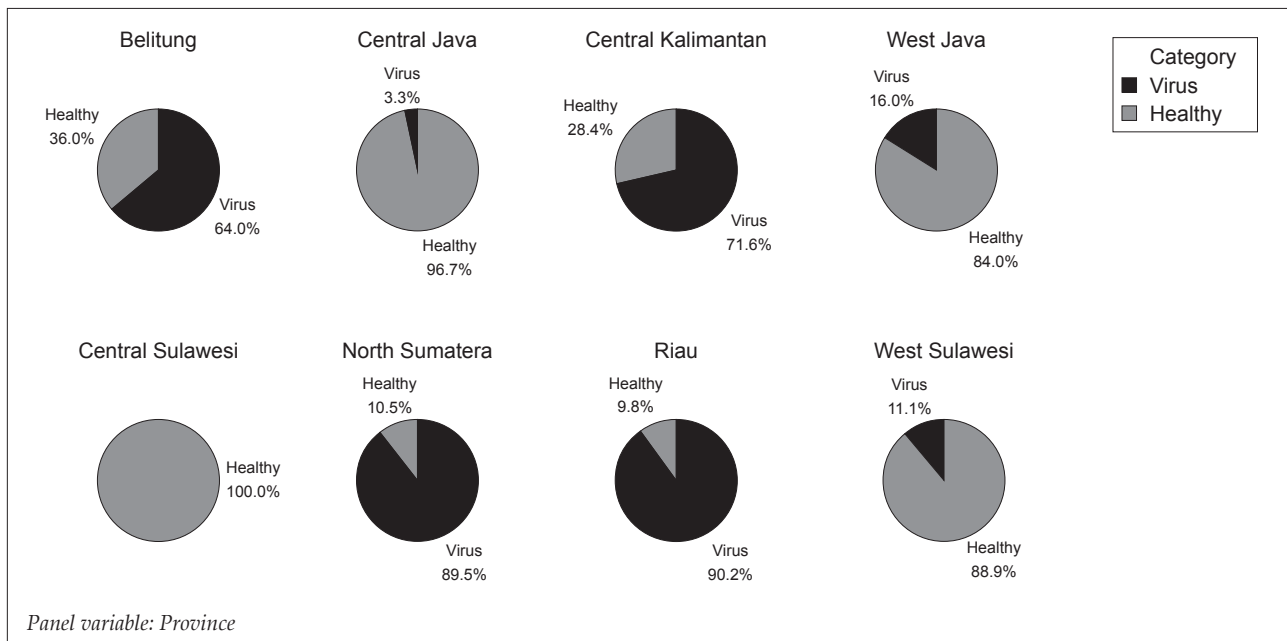


Figure 7. Nudivirus (OrNV) infection rates in adult Indonesian *O. rhinoceros*.

Number of Eggs in Infected Females

Healthy females *O. rhinoceros* and those infected by OrNV have almost the same number of eggs, 128 and 122. Of those females with OrNV infection, 57% had eggs in their abdomens (Figure 8). Females *O. rhinoceros* from oil palm intensive cultivation areas, i.e. Sumatra, Belitung and Kalimantan still produced eggs even when they were infected by OrNV (Figure 9). While, infected females *O. rhinoceros* from Java and Sulawesi tended not to have eggs (Figure 10).

Although there were high OrNV incidence in Sumatra, Belitung and Kalimantan, *O. rhinoceros* still survived in good reproductive condition. Zelazny and Alfiler (1991) stated that OrNV was an important control of *O. rhinoceros* in South-east Asia, but *O. rhinoceros* outbreaks continue to occur even though some of the individuals in populations are infected. Evidence suggests that the extensive availability of organic material at replanting is the reason for *O. rhinoceros* outbreaks (Chenon and Pasaribu, 2005; Salim and Hosang, 2013). The OrNV may have had significant opportunities to change its genetics and circulate to other abundant hosts. Crawford and Zelazny (1990) stated that OrNV genomics always changed with nonlethal infections.

OrNV is a species in the genus *Alphanudivirus*, family *Nudiviridae* (Jehle *et al.*, 2013). Nudivirus adopts with vertical and horizontal modes of infection (Williams *et al.*, 2017). Vertical infection is also known as latent or persistent infection (Wang and Jehle, 2009) and changes the virus to become asymptomatic. Latent infections burst into active depending on host environmental resistance or host physiological stresses. Activated viruses from

a symptomless condition cause lethal infections and eventually horizontal transmission occurs (Cory, 2015). It is suspected that most Indonesian *O. rhinoceros* populations from oil palm intensive cultivated areas are persistently or latently infected by OrNV and the beetles remain fertile.

Adults Attracted by Pheromone and Size of the Adults

Pheromones attracted more females than males, with the highest percentage of 77.2% in North Sumatra and data average from eight sampling sites was 61.5% (data not shown). Other research has also indicated that females are more strongly attracted by pheromones than males, with results of 68% in India, 60% in Malaysia and 81% in North Sumatra. These females may have been searching for mates or seeking breeding sites (Bedford, 2014; Zelazny and Alfiler, 1991; Morin *et al.*, 1996). Attracted females may come from breeding sites or the crowns of oil palms. Emergent adults fly to the crowns of palms for five weeks then fly to lay eggs in breeding sites where they stay for seven weeks, before returning to drill into oil palm crowns to feed (Norman and Basri, 2004).

Both male and female *O. rhinoceros* had a median length of 4 cm. First quartile (Q1) female length was 3.8 cm, with 4.3 cm for third quartile (Q3). Q1 male length was 3.7 cm, with 4.2 cm for Q3. Adult length is dependent on nutritional values during larval phases (Pallippambal, 2015). The number of female *O. rhinoceros* that is attracted by pheromone and the adult size captured from field seem not to be affected by the presence of OrNV infection.

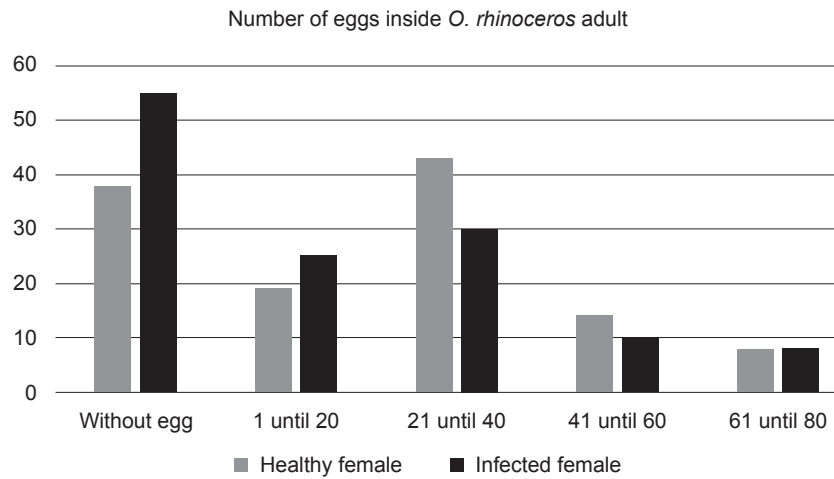


Figure 8. Number of eggs inside the *O. rhinoceros* adult.

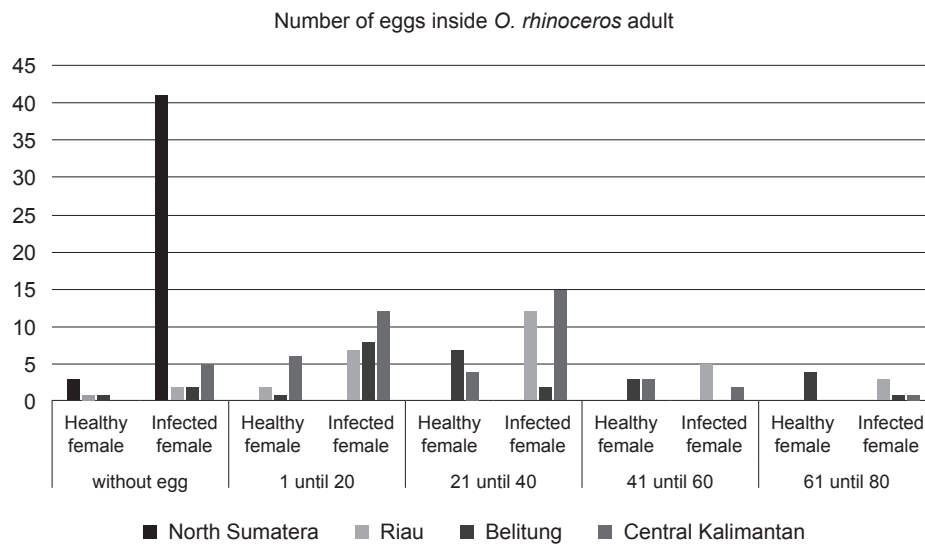


Figure 9. Female *O. rhinoceros* from Riau, Belitung and Central Kalimantan were produced eggs although infected by Nudivirus (OrNV).

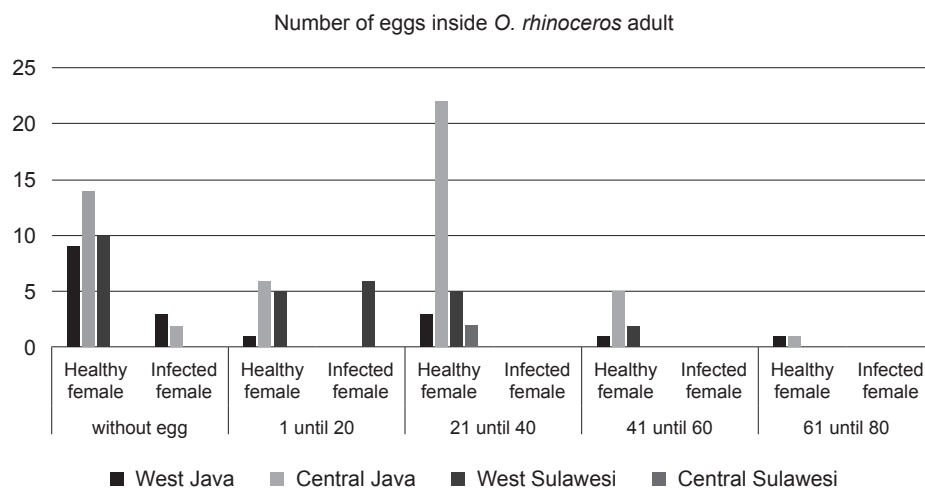


Figure 10. Infected female *O. rhinoceros* from West Java, Central Java, West Sulawesi and Central Sulawesi tended not to produce eggs.

CONCLUSION

OrNV infected larvae can be identified by external body characteristics, while infected adults can be identified by internal identification of whitish, swollen midgut. High incidence of OrNV infection in *O. rhinoceros* can be correlated with intensive oil palm cultivation such as found in Sumatra, Belitung and Kalimantan. In some cases, infected adults from Sumatra, Belitung and Kalimantan were found to be alive and with eggs in their abdomens.

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