

Some Notes on Biological Aspects of Captive Javan Warty Pig (*Sus verrucosus*)

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ABSTRACT

The Javan warty pig (*Sus verrucosus*) is an endemic pig to Java and Bawean Islands, while population on Madura Island is thought to be extinct. The problem in establishing ex-situ captive breeding is the lack of information on biology or physiology. A study on these aspects was conducted in 16 Javan warty pigs and 2 cadavers in Surabaya Zoo, Surabaya. Birth profile was evaluated and blood collections were conducted, as well as analysis on spermatozoa morphology. Data showed that blood parameters were not different among the age groups (juvenile and adult) or sex and within the range of *Sus scrofa*. Extreme values were only obtained from the palette with the female reaching $14.5 \times 10^3/\text{mm}^3$, while adult male and juvenile pigs were $58-75 \times 10^3/\text{mm}^3$. Diameter of both testicles with skin intake was 56.42 mm, with the length of left testicle being 83.29 mm and right testicle 78.88 mm. Javan warty pig spermatozoa had longer size for the head and tail lengths compared to average pigs sperms. Litter size was between two to four, with the average of 2.75 litters (SD 0.98). Low litter size in this species is something that has to be concerned from the conservation point of view, therefore a captive breeding population program needs to be considered.

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Key words: Javan warty pig, *Sus verrucosus*, blood parameters, sperm.

INTRODUCTION

The Javan warty pig (*Sus verrucosus*) is an endemic pig to Java and Bawean Islands, while the population on Madura Island is thought to be extinct (Semiadi and Meijaard, 2006). The species is believed to have emerged during the Pleistocene era, almost two millions years ago. Taxonomically, there are still some disagreements on the relation among other pig species (Suripto, 2002; Lucchini et al. 2005), however distinct characters occurred between the Javan warty pig with wild pig *Sus scrofa* (Suripto, 2002).

Presently, in Indonesia, the species is not protected, however at International level, the IUCN Species Survival Commission has put *S. verrucosus* in 'endangered' category (IUCN, 2007). This shows that the species needs priority attention on conservation action regardless of its non protected status under the Indonesian law. To some level, the species has often been blamed as pest due to its stereotype behavior with wild pig *S. scrofa*.

Last field survey indicated that the distribution of the species is now fragmented into several small pockets habitat with unknown size of population. Hence, it is believed the population number has decreased drastically since the past study (Semiadi and Meijaard, 2003; 2006). Those decreases are mainly due to hunting activities in the past 10-20 years ago, along with the expansion of the agricultural lands and production forests. Therefore, a conservation effort is needed to save the species through the development of ex-situ facilities with target populations for re-introduction programs.

The main problem in ex-situ development is the limited information on the biology and physiology of the species. Therefore, some observations taken from captive animals were conducted with the aim to gain more information on the biology or physiology of the Javan warty pig.

MATERIALS AND METHODS

The study was conducted in Surabaya Zoo, Surabaya, East-Java, using captive Javan warty pigs (*Sus verrucosus*) that were put in several exhibition and quarantine pens. The majority of the animals were born in captivity, and only two animals were traceable for their origins.

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Birth profile

Birth profile was evaluated from the Surabaya Zoo database with the calculation on mean birth date followed Semiadi et al. (1994). Dates of birth were transformed to numerical sequence in Excel (Microsoft Excel 2003) and calculated the mean and standard deviation, and then re-transformed to the actual date.

Blood collection

Blood collection was conducted in 16 (= 9; = 7) mixed ages (juvenile, adult, old) Javan warty pigs which were captured using net. Blood was collected either from jugular vein or fore-legs/hind-legs vein, using 5 cc syringe with a 0.5 inch x 20G needle. Collected blood was split into two portions, half of which was transformed into blank vials, and the other half into vials filled with ethylene diaminetetraacetic acid (EDTA). Within two hours after the collection, all blood samples were transported to the designated Surabaya Zoo Medical Laboratory (Laboratorium Medik Prodia) for analysis according to a protocol that has been set and calibrated for wildlife animals. Hematological analysis was run on Advia.12.0 seri 411.OT76011 (USA) and biochemical analysis was run on Modular P.800 (Roche, France).

Since the sample numbers were considered minimal, data were pooled and analyzed descriptively.

Spermatozoa morphometry

Semen was collected from cauda epididymis through aspiration using 10 µL micropipette in one adult cadaver. Five µL of collected semen was then diluted into 995 µL buffer formolsaline solution and kept in tight vials. In the Reproduction Laboratory, Research Centre for Biology LIPI, Cibinong-Bogor, semen was processed as wet mount and evaluated under phase contrast microscope at 40x magnification (Nikon Optiphot-2), attached to a Nikon FDX 35 camera for documentation. The photos were then scanned at 600 dpi. resolution (Canon 3000 F) and analyzed using Image J ver. 3.7 program.

To transform the pixel value into metric unit, one pixel produces by the Image J ver. 3.7 program was calibrated using micrometer photo at similar magnification, resulting

an equivalent value of 9.3 pixel/micrometer. Spermatozoa parameters and area analysis followed Kondracki et al. (2005), whereas spermatozoa head total area was calculated following Révay et al. (2004).

Apart from one adult cadaver, one male juvenile (1.5 months) Javan warty pig cadaver was also inspected for any specific characters. All data were processed in Excel (Microsoft Excel 2003) followed by simple statistic calculations.

RESULTS AND DISCUSSION

Blood parameters analysis revealed that no big variations exist among sex or age groups. The only extreme value was on a palette parameter (PLT, $10^3/\text{mm}^3$), in which female group had a value of $14.5 \times 10^3/\text{mm}^3$, compared to the adult male and juvenile groups with only $58\text{-}75 \times 10^3/\text{mm}^3$. Since the best normal parameters of blood values of any species collected should be on specific sex and age categories, the current data are meant as a general guidance only until more data are obtained. In general, the range of each parameter was not too

Table 1. Comparisons on blood hematological and biochemical parameters of several pig species.

| Parameters | <i>S. verrucosus</i> ^a | <i>S. domesticus</i> ^b | <i>S. domesticus</i> ^c | <i>S. scrofa</i> ^c | <i>S. s. aferus</i> ^e |
|-------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|-------------------------------|----------------------------------|
| Tot Prot (g/dL) | 5.80-10.30 | 5.83-8.32 | 6.5-9.0 | 7.7±0.58 | 8.21±0.38 |
| Albumin (g/dL) | 2.30-5.50 | 2.26-4.04 | 31-43 | 4.3±0.36 | 4.08±0.29 |
| Globulin (g/dL) | 3.30-6.90 | 3.95-6.00 | - | - | - |
| WBC ($10^3/\text{mm}^3$) | 6.10-7.87 | 11-22 | 10.6-24.0 | 11.5±3.24 | 9.79±4.27 |
| RBC ($10^6/\text{mm}^3$) | 7.94-8.90 | 5-7 | 5.1-8 | 5.7±1.06 | 8.0±0.68 |
| HGB (g/dL) | 15.90-18.80 | 9-13 | 10-17 | 12.0±2.15 | 15.7±1.73 |
| HCT (%) | 54.60-68.80 | 36-43 | - | - | - |
| PLT ($10^3/\text{mm}^3$) | 12.00-75.00 | 200-500 | - | 310 | - |
| MCV (μm^3) | 65.20-77.40 | 52-62 | 52-63 | 63.0±4.04 | 77.5±5.13 |
| MCH (pg) | 18.40-22.00 | 17-24 | 18-22 | 21.0±1.38 | - |
| MCHC (g/dL) | 27.10-32.20 | 29-34 | 34-380 | 33.5 | - |
| CHCM (g/dL) | 27.20-30.70 | - | - | - | - |
| RDW (%) | 16.00-17.20 | - | - | - | - |
| HDW (g/dL) | 1.83-2.41 | - | - | - | - |
| MPV (μm^3) | 8.00-11.70 | - | - | - | - |
| PDW (%) | 7.00-68.30 | - | - | - | - |
| PCT (%) | 0.01-0.07 | - | - | - | - |
| LI (%) | 0.00-2.39 | - | - | - | - |
| NEUT (%) | 22.40-37.50 | 20-70 | 15.1-59.5 | - | - |
| LYM (%) | 11.30-23.10 | 35-75 | 25.5-71.1 | - | - |
| MON (%) | 42.30-51.90 | 0-10 | 1-14 | - | - |
| EOS (%) | 0.00-0.60 | 0-15 | 1-13 | - | - |
| BASO (%) | 0.80-15.30 | 0-3 | 0-3 | - | - |
| LUC (%) | 0.70-3.50 | - | - | - | - |
| # NEUT ($10^3/\text{mm}^3$) | 1.37-2.48 | 2-15 | 1.9-10.1 | 5.7±2.90 | - |
| # LYM ($10^3/\text{mm}^3$) | 0.75-1.67 | 3.8-16.5 | 3.7-14.7 | 5.3±2.29 | - |
| # MON ($10^3/\text{mm}^3$) | 3.11-3.76 | 0-1 | 0-2.4 | 0.203 | - |
| # EOS ($10^3/\text{mm}^3$) | 0.00-0.04 | 0-1.5 | 0-2.4 | 0.139 | - |
| # BASO ($10^3/\text{mm}^3$) | 0.06-0.94 | 0-0.5 | 0-0.5 | 0.150 | - |
| # LUC ($10^3/\text{mm}^3$) | 0.06-0.23 | - | - | - | - |
| BLAST (%) | 0.10-1.20 | - | - | - | - |

Note: ^a Present data; ^b Merck (2006); ^c Brockus et al. (2005); ^d Friendship et al. (1984); ^e Harapin et al. (2003).

different from *S. scrofa* and domesticated pigs (Table 1). The only parameters which gave distinct differences with other pig species were on hematocyte, lymphocyte and monocyte.

Testicle shape was similar to common pig species, with the diameter of both testicles with skin intake was 56.42 mm, and length for the left part was 83.29 mm and right part was 78.88 mm. Specific character was observed on spermatozoa where head and tail length tended to be longer compared to *S. scrofa* or *S. s. domesticus* (Tables 2 and 3). High cytoplasmic droplet was found in almost all spermatozoa being evaluated (Figure 1). Cytoplasmic droplet is a remnant of the germ cell cytoplasm, that remains adherent at the neck region of the elongating spermatid when it is shed as a testicular spermatozoon at spermiation during normal spermatogenesis (Cooper, 2005). In boar and goat, spermatozoa from the cauda epididymis have a high percentage of distal cytoplasmic droplets that can reach more than 93% (Kato et al., 1996). Whereas in the Javan warty pig, the majority cytoplasmic droplets observed were found at midpiece reaching 97%.

Table 2. Sperm morphometry of adult *S. verrucosus*.

| | Head | | | Tail |
|--------|-------------------------|--------------------------|--------------------------|--------------------------|
| | Width (μm) | Length (μm) | Area (μm^2) | Length (μm) |
| Means | 4.86 | 9.95 | 38.54 | 48.01 |
| Stdev. | 0.433 | 0.488 | 3.234 | 2.420 |
| Max. | 7 | 11 | 32 | 53 |
| Min. | 3 | 8 | 46 | 36 |

Carpal-gland which was located posteriorly on the fore-legs consisted of four lobes that were arranged in line. (Figure 2). The structure of the carpal gland had been visible since 1.5 months old, but with smaller size compared to that the adult ones. In adult pig, the distance between lobes varied from 9.00 mm to 14.75 mm, and the diameter from 0.15 mm to 0.30 mm, from the lower to the top position, respectively. The function of the lobes gland was believed as scent gland in the defensive behavior of reproductive females or as a communication strategy as it is shown in *Sus domesticus* (Heise-Pavlov, 2005). The number of lobes in this study was less than other Suidae which has five to seven lobes as stated by Farnesi et al. (1999).

Hoof size of adult pig was shown in Table 4, whilst warts and body size was shown in Tables 5 and 6. Necropsy of fresh warts showed no fat deposit and the tissue was firm. Body size of adult cadaver was within the range of common pig species such as *S. scrofa* (body length 128.7 cm, body weight 56.7 kg, tail length 19.6 cm, ear length 13.1 cm and age 13-18 months; Moretti, 1995).

Three years birth profile (2003-2005) showed a mean birth date of 01 May (SD 60.57 days, $n=4$), with the range of birth month from March to August. Litter size was considered low, only 2-4 individuals, with the mean 2.75 individuals (SD 0.98, $n=4$). No sex ratio was able to determine. Local hunters in West Java



Figure 1. Spermatozoa of adult Javan warty pig (*S. verrucosus*) at 40 x magnification, using phase contrast microscope. Bar= 25 μm (Photo: R.T.P Nugraha).



A



B

Figure 2. Gland lobes on fore-legs of adult (A) and juvenile (B) Javan warty pig *S. verrucosus* (Photo: G. Semiadi).

Table 3. Comparison of spermatozoa morphometry in several pig species.

| Species | References | Head | | | Tail |
|------------------------|-------------------------|-------------|-------------|-------------------------|--------------|
| | | Width (µm) | Length (µm) | Area (µm ²) | Length (µm) |
| <i>S. verrucosus</i> | Present study | 4.86 ± 0.43 | 9.95 ± 0.49 | 38.54 ± 3.23 | 48.01 ± 2.42 |
| <i>S. scrofa</i> | Gage (1998) | 5.0 | 8.1 | -- | 28.8 |
| <i>S. s. domestica</i> | Hirai et al. (2001) | 4.66 ± 0.02 | 9.27 ± 0.05 | 35.7 ± 0.22 | -- |
| <i>S. s. domestica</i> | Kondracki et al. (2005) | 4.88 ± 0.28 | 9.37 ± 0.44 | 41.17 ± 2.68 | 46.26 ± 1.75 |

noted that litters size of wild Javan warty pigs was 50% less of that the *S. scrofa* (Semiadi, 2005; unpublished data). Coblenz and Baber (1987) reported that litter size of *S. scrofa* was between 1-10 individuals, with the mean of 4.7 individuals and mortality rate at 19 weeks old reaching 52%. Whereas Baber and Coblenz (1986) showed the mean of litter size for several pig species were between 4.2-7.4 individuals, with post weaning mortality reaching 12.5-34.7%. This shows that in Javan warty pig, low litter size could be a limitation on the success of captive breeding for the re-introduction program within a short period of time, unless excessive numbers of founders are available.

Table 4. Hoof measurements of adult male Javan warty pig *S. verrucosus*.

| | Fore-legs | | Back-legs | |
|-------------|-----------|-------|-----------|-------|
| | Left | Right | Left | Right |
| Length (mm) | 46.74 | 43.96 | 44.67 | 41.97 |
| Width (mm) | 22.28 | 22.28 | 19.39 | 18.83 |

Table 5. Warts size of adult male Javan warty pig *S. verrucosus*.

| | Lateral (Length) | Distal (Width) | |
|---------------------|------------------|----------------|-------|
| Under the eyes (mm) | 28.56 | 22.43 | |
| On cheek (mm) | 120.45 | Close to eyes | 39.45 |
| | | Centre | 15.27 |
| | | Bottom | 23.40 |
| On nose (mm) | 37.72 | 21.88 | |

Table 6. Body measurements of adult male Javan warty pig *S. verrucosus*.

| Parameters | Size (cm) |
|---------------------------------|-----------|
| Body length (nose to tail bone) | 125.0 |
| Tail length | 19.5 |
| Girth diameter | 87.0 |
| Ear length | 10.95 |

CONCLUSIONS

From this study it was shown that normal blood parameters of Javan warty pigs were within the range of commonly found pigs. However, the pig had low number of litter per birth.

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