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Research

Rapid Survey for Population, Commercial Trade of Small-Clawed Otter (*Aonyx cinereus* Illiger, 1815) in Java and Preliminary Assessment of Potential Bacterial Zoonoses

Endah Dwijayanti[1*], Nurul Inayah[1], Wartika Rosa Farida[1], Eko Sulistyadi[1], Sugiyono Saputra[2]

[1]Museum Zoologicum Bogoriense, Research Center for Biology, Indonesian Institute of Sciences, Bogor, Indonesia
[2]Microbiology Division, Research Center for Biology, Indonesian Institute of Sciences
*Corresponding author: endahdwijayanti1@gmail.com
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ABSTRACT

Small-clawed Otter (*Aonyx cinereus*) is the smallest Indonesian otter species commonly traded as a pet. This species is listed in CITES appendix I and is still not protected in Indonesia. This study investigates the species' illegal trade in the local market and assesses potential bacterial zoonoses transmitted to pet buyers. In general, we did not find *A. cinereus* trade in a traditional market in both East Java and Central Java. This fact is inversely proportional to the high online transaction. A rapid survey on the potential habitat of Small-clawed Otter in Central Java shows that suitable habitat is available, and we found many field signs (feces, traces, and nest) of Small-clawed Otter. The preliminary assessment on potential bacterial zoonoses conducted by isolating bacteria from anus, mouth and skin in selective medium for Enterobacteriaceae. Molecular identification using 16S rRNA showed that several species of bacteria such as *Citrobacter freundii* (n=3), *Proteus alimentorum* (n=2) and *Klebsiella pneumoniae* (n=1) were commonly observed. Based on this research, further study is still needed. We suggest that illegal trade need monitoring to reduce harvesting activities in the wild, and the biological risk of capturing and owning Small-clawed Otter as a pet.

Keywords*: Aonyx cinereus*, Small-clawed Otter, commercial trade, bacterial zoonoses

ABSTRAK

Berang-berang Cakar Kecil (*Aonyx cinereus*) adalah jenis berang-berang Indonesia terkecil yang biasa diperdagangkan sebagai hewan peliharaan. Spesies ini masuk dalam CITES appendix I dan masih belum dilindungi di Indonesia. Studi ini menyelidiki perdagangan ilegal spesies tersebut di pasar lokal dan menilai potensi zoonosis bakteri yang ditularkan kepada pembeli hewan peliharaan. Secara umum kami tidak menemukan secara langsung perdagangan *A. cinereus* di pasar tradisional baik di Jawa Timur maupun di Jawa Tengah. Fakta ini berbanding terbalik dengan tingginya transaksi online. Selanjutnya, survei terhadap potensi habitat Berang-berang Cakar Kecil di Jawa Tengah menunjukkan bahwa masih tersedia habitat yang sesuai, yang ditunjukkan dengan banyak tanda keberadaan (kotoran, jejak, dan sarang) Berang-berang Cakar Kecil. Studi awal terhadap potensi bakteri zoonosis dilakukan dengan mengisolasi bakteri menggunakan media selektif untuk Enterobacteriaceae. Identifikasi molekuler dengan 16S rRNA menunjukkan bahwa beberapa spesies bakteri seperti *Citrobacter freundii* (n = 3), *Proteus alimentorum* (n = 2) dan *Klebsiella pneumoniae* (n = 1) banyak ditemukan. Berdasarkan penelitian tersebut masih diperlukan studi lanjutan. Kami menyarankan bahwa perdagangan ilegal perlu pemantauan untuk mengurangi aktivitas pemanenan di alam liar, dan risiko biologis dalam menangkap dan memiliki Berang-berang Cakar Kecil sebagai hewan peliharaan.

Kata kunci*: Aonyx cinereus*, Berang-berang Cakar Kecil, perdagangan, bacterial zoonosis

INTRODUCTION

The Small-clawed Otter (*Aonyx cinereus* Illiger, 1815) is one of the 13 species of otter in the world. Four species found in Indonesia, namely Smooth-coated otter (*Lutrogale perspicillata*), Eurasian otter (*Lutra lutra*), Hairy-nosed otter (*Lutra sumatrana*), and Small-clawed Otter (*Aonyx cinereus*). The Small-clawed Otter is the smallest type of otter, body length ranging from 65-75 cm and weighing about 2-6 kg. The tail is tubular conical to the end with a length of about 24-30 cm. The pelage is brown, sometimes cream or slightly reddish. The tips of the body hair are greyish, which is the origin of the word cinerea. The lower body is pale brown, the upper lip, chin, throat, and sides of the neck and face are grey and white (Larivière, 2003). The limbs are short and have a swimming membrane, but not entirely closed to the fingers' tips. The fingers have short claws that do not stick out from the fingers' tips, except when Otters are very young. The shape of the hairless rhinarium is like a "V" at the top (Pocock, 1941). *A. cinereus* possess two pairs of mammary glands (Wright, 2015). Female of *A. cinereus* reaches sexual maturity around two years, and male around 2,8 years. The gestation period is approximately 60 to 64 days. Mated pairs can have 2 to 7 litter per year (Maharadatunkamsi *et al*., 2020).

So far, the population data for *A. cinereus* is still limited, but it is suspected that there has been a decline in the population in many areas of its range (Hussain *et al*., 2011). In other hands, adorable features and trainable cause high demand for juvenile otters as a pet. Several studies reported otter illegal trading and seizure across Asia (Yoxon & Yoxon 2015; Siriwat & Nijman 2018; Gomes & Bouhuys 2018; Harrington *et al*., 2019). Gomez & Bouhuys (2018) reported that in 2016-2017, 59 live otters seized from Thailand with trade destination were Japan and Vietnam. Also, 560 advertisements from January to April 2018, 960 otters observed to sell in online trade. *A. cinereus* listed in IUCN red list as Vulnerable. Combined with habitat loss and poaching, this species population's keeps declining year by year (Wright *et al*., 2015). The high demand for otters in the pet industry will increase the threat to the conservation of Asia's wild otters, requiring strict international regulation and increasing enforcement efforts to crack down on the illegal trade in otters in Asia (Gomes & Sheperd 2019). The Animal Committee (CoP18 CITES) meeting in Geneva 2019 decided that Asian Small-clawed Otter listed in CITES Appendix I. It implies international commercial trade for this species is restricted only under exceptional circumstances. The uplisting resolution did not directly influence domestic trade because the Small-clawed Otter still not protected in Indonesia. Aadrean (2013) revealed the most widely traded (83.9 %) online is *A. cinereus,* and all of the trading were recognized from Java island, with East Java province is the most prominent trade activity (34,9%). Recently, online trade monitoring has shown that hundreds of juvenile sale in Indonesia, and evidence suggests it taken from the wild (Gomez *et al*., 2019).

Wildlife has a wide range of ecological, economic, and cultural importance concerning human existence. Nevertheless, on the other side, The majority of global emerging infectious diseases (EIDs) 70% (Can *et al*., 2019) to 71.8% (Zeigler & Engel, 2020) originate in wildlife. Zoonoses with a wildlife reservoir are typically caused by various bacteria, viruses, and parasites, whereas fungi are of minor importance (Kruse *et al*., 2004). Pavlin *et al*.(2009) revealed from a total of 246,772 mammals in 190 genera (68 families) that imported to the USA, genera capable of harbouring the most significant number of risk zoonoses were Canis and Felis (14 each), Rattus (13), Equus (11), and Macaca and Lepus (10 each). There is still no information about the potential zoonoses from *A. cinereus*. However, the high demand for the species as pets can also escalate exposure to zoonotic disease in humans. The ownership of exotic pets are proven to transmit pathogens such as *Salmonella* spp., *Leptospira* spp., *Bartonella* spp., *Mycobacterium* spp., *Cryptosporidium parvum*, and many more through ingestion, inhalation, infected animal scratches, animal bites, contact with infected animal tissue, animal urine, and animal feces (Souza, 2009).

We study the otter trade in the animal market in East Java through this research, investigate potential habitat in Central Java and determine the zoonotic potential transmitted from the Small-clawed Otter.

MATERIAL AND METHODS

Survey Location

The survey carried out in East Java from 26 April to 2 May 2019 and from 18 to 22 August 2019 in Central Java. We surveyed Small-clawed Otter trade in three cities' markets (Surabaya, Malang, and Banyuwangi) in East Java and three towns (Semarang, Batang and Kendal) Central Java. Microbial sampling carried out in captive breeding facilities in East Java. The survey location showed in Figure 1.

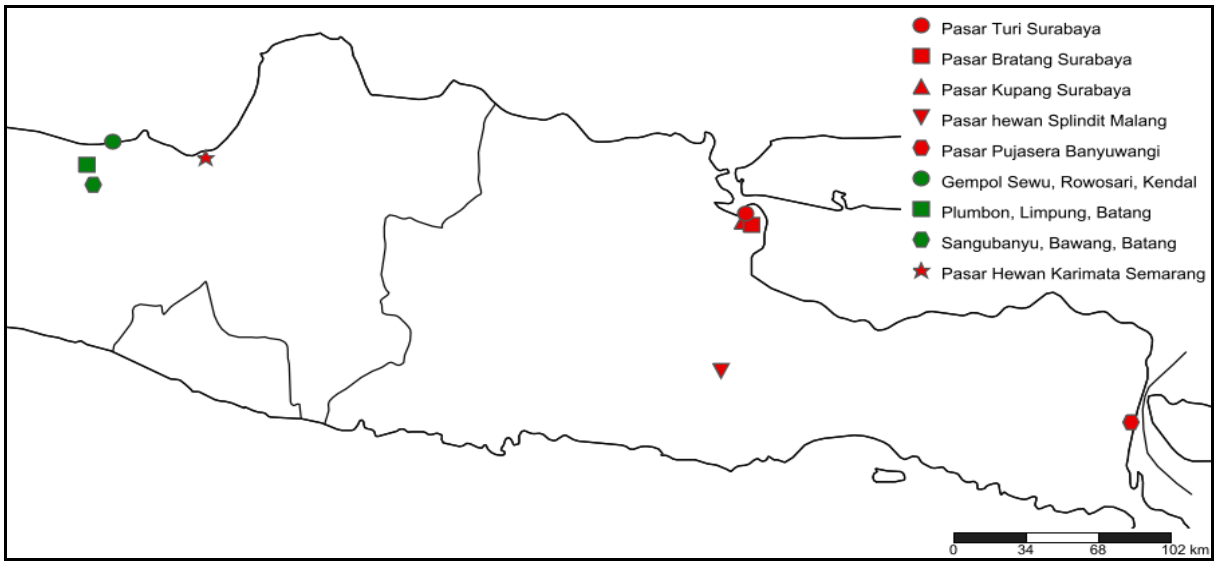

Figure 1 Survey location for Small-clawed Otter in Java

Market Survey

The method used in the trade survey is direct visits to animal markets and interviews with wildlife traders using the snowball technique and survey activities photographed.

Habitat Survey

To survey the potential habitat of *A. cinereus*, we gathered information from the Natural Resources Conservation Agency (BKSDA) of Central Java. The presence of Small-clawed Otter is identified in the field based on field signs (nest, traces, and feces). All survey data were analyzed descriptively.

Assessment of potential zoonoses

Potential zoonoses were assessed using the culture-dependent method. Bacterial sampling was carried out by swabbing the anus, mouth and hands, then cultured in different selective media, namely *Salmonella-Shigella* Agar (SSA) and Mac Conkey Agar (MAC), to detect the presence of pathogenic Enterobacteriaceae, especially *Salmonella*. Several colonies with various appearance, including suspected *Salmonella,* were preserved and identified based on 16S rRNA gene. Bacterial DNA was extracted using the Genomic DNA Mini Kit (Genaid), and the 16S rRNA gene was amplified using Veriti 96-Well Thermal Cycler (Applied Biosystems) before subjected for dideoxy sequencing (1stBaseAsia, Singapore). Sequences were analyzed using Bioedit software dan compared to the databases using the BLAST program in GenBank®.

RESULTS AND DISCUSSION

Market Survey

The survey result showed that Small-clawed Otters traded in 5 animal markets in East Java and one animal market in Central Java. Only one animal market (Pasar Pujasera in Banyuwangi) provided live otters. The results of the survey in several animal markets are presented as follows:

1. Pasar Bratang, Surabaya

Pasar Bratang is the biggest animal market in Surabaya. During the survey, Small-clawed otter was not encountered in this market. However, two dealers could provide otter by request. Usually, they will contact their hunter client to hunt otters in the wild and sell it as either living or bushmeat—the origin for Small-clawed Otter in this market mainly from Madura, Jember, and Kediri. The hunter could harvest up to 6 (six) individuals. But the hunter needs 1-2 weeks to fulfil the demand because it was challenging to obtain the otters at one time in the exact locations. Also, otters are aggressive and have a good defense while they are threatened. Hunter usually using the particular net to catch otter in the night. Juvenile individual worth IDR 500.000, more expensive than older individuals worth IDR 200.000 – 300.000.

2. Pasar Turi, Surabaya

Pasar Turi Surabaya is smaller than Pasar Bratang Surabaya. We could not encounter Small-clawed Otter in Pasar Turi Surabaya, neither otter dealers. Based on an animal trader interview, a dealer could provide 11 individuals of juvenile otter but no further information about the dealer contact. The otters usually originated from Bangkalan, Madura.

3. Pasar Kupang, Surabaya

As in Pasar Turi, Small-clawed Otter or the dealers were not found in Pasar Kupang. This market is smaller than the two markets mentioned above and not open every day. This market looks more like a temporal market than a permanent one. Based on an interview with a former wildlife trader, this market sells many kinds of animals in the past. Now, this market only sold domesticated and unprotected animal such as commercial bird.

4. Pasar Splindit, Malang

Pasar Splindit is a center animal market in Malang. It is the only market that put prohibition sign of trading protected and endangered animals (Figure 2). We did not find *A. cinereus* in this market. Several informants have confirmed that the otter trade in Pasar Splindit Malang is rare. They presume that all otter species in Indonesia are protected by law. Once the otter was available, only a single individual, usually a juvenile stage, because it was easy to train than an adult. In this market, one young individual worth IDR 500.000, the same as the otter price in Pasar Bratang Surabaya. There are two types of sources in Pasar Splindit Malang, capture in the wild and illegal breeder. Hunter usually using a hunting dog to capture the otter. However, the informant not willing to reveal further information about either the hunter or the breeder.

5. Pasar Pujasera, Banyuwangi

We found one individual of juvenile Small-clawed Otter in Pasar Pujasera Banyuwangi (Figure 3). The juvenile sells for IDR 200.000—the baby otter is in good condition. The trader has no information about the individual's origin because he bought it from another trader. The trader informs that otter have less buyer than another common wild pet like the civet. Another transaction revealed from another trader an otter worth IDR 250.000 – IDR 300.000. Usually, it has obtained from Banyuwangi.

6. Pasar Karimata, Semarang

There is no sign of otter trade in Pasar Karimata Semarang. We did not encounter both the living otter and the dealers. However, we find Tupai kekes (*Tupaia javanica*), Long-tailed Macaque (*Macaca fascicularis*), Asian Palm Civet (*Paradoxurus hermaphroditus*), Asian water monitor (*Varanus salvator*), Barn Owl (*Tyto alba*), Tokay Gecko (*Gekko gecko*), and Bats (Chiroptera) (Figure 4).

Small-clawed Otter Trade

The survey results show the difficulty of encountering Small-clawed Otter in the six markets. Many market surveys across Indonesia have been held by Shepherd (2012) in the four largest wildlife markets in Jakarta. Nijman & Nekaris (2014) carried a study in Bali's three markets and did not find any Small-clawed Otter. The widespread use of social media as the online market is probably becoming one reason animal trader no longer sells on the traditional animal market. Social media is more efficient because it offers convenience, accessibility, and privacy for any animal trader to sell many exotic pets, including Small-clawed Otter (Siriwat & Nijman, 2018) compared to trade in the animal market directly.

Figure 2 Prohibition sign for trading protected and endangered animals in Pasar Splindit Malang

Figure 3 Baby Small-clawed Otter sell in Pasar Pujasera Banyuwangi

Figure 4 Mammals, bird and reptile traded in Pasar Karimata Semarang. (A) *Paradoxurus hermaphroditus*, (B) Bats, (C) *Gekko gecko*, (D) *Varanus salvator*, (E) *Tyto alba*, (F) *Tupaia javanica*, (G) *Macaca fascicularis*

We found a similar pattern in the small-clawed otter trade. The individual Small-clawed Otter are not available directly on the market, but traders are willing to provide otters upon order. Some traders showed the same photo when offering Small-clawed Otter. Some of these traders are possibly connected with the same hunter or have the same trading network.

Most of traded Small-clawed Otter was obtained by poaching. Animals captured from many East Java regions such as Jember, Kediri, Banyuwangi, and Madura. Unfortunately, there is no information about populations and distribution–in these locations or any nearby location.

The demand for Small-clawed Otter commonly an exotic pet. From all market survey, only one informant in Pasar Pujasera Banyuwangi uses this species as bushmeat. No study reported the Small-clawed Otte r as bushmeat. However, consuming otter as bushmeat reported in another sister taxa, *Aonyx congicus* (Jacques *et al*., 2009), and *Aonyx capensis* (Angelici *et al*., 1999). Alves *et al*. (2013) reported *Aonyx cinereus* and other mustelids used as a traditional medicine in Southeast Asia. *Aonyx cinereus* used as liver and kidney traditional medicine in Cambodia and Lao PDR.

Prices offered from all the markets surveyed are relatively the same, ranging from IDR 200,000-500,000. Adult Small-clawed Otters are less expensive than juveniles. The reason is the young *A. cinereus* are still easily tamed and trained. Prices in online trading vary between IDR 150,000 - 800,000 (Aadrean, 2012).

Predicting Potential Habitat

We observed 6 (six) location as potential habitats of *A. cinereus* in Kendal, Batang, and Semarang, Central Java Regency. We have observed a fishpond in the

coastal zone in one site in Kendal. We also observed five areas, including several rivers in Batang. Based on the visual observation, we did not find *A. cinereus*, but signs of its existence are indicated by the presence of former nest, traces and feces. Local people who live around the river confirmed a group of *A. cinereus* usually appears in the night foraging for food in the river or fishpond. The prediction of the potential habitat of *A. cinereus* showed in Table 1.

Potential Population

The Small-clawed Otter has a wide distribution in Asia. However, information on the existence of the Small-clawed Otter in Indonesia is still limited. So far, IUCN has reported *A. cinereus* in only a limited number in West Java, Central Java, and East Java. The survey shows that in Central Java, there are still quite a lot of Small-clawed Otter habitat spots scattered in Semarang, Batang and Kendal. It can be predicted that this species present is also spread in almost all Central Java districts with landscape conditions that match *A. cinereus* habitat description (Table 1).

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The Small-clawed Otter lives in freshwater forests and peat swamps, rice fields, lakes, rivers, reservoirs, canals, mangrove forests, and coast (Sivasothi & Nor 1994; Huda *et al*. 2018). Observations show that in Central Java, the species can be found in a pond or coastal, rocky and shallow river flows, residential areas with fish ponds and rice fields. The results of potential habitat observation at several locations as the previous study revealed that *A. cinereus* could be found in Indonesia's coastal wetlands and rice fields. This fact also indicates that *A. cinereus* can adapt to the climate in tropical South and Southeast Asia (Melisch *et al*., 1996). Residents confirmed that *A. cinereus* lives in groups consist of 10-20 individuals. This number is more prominent than the previous study by Wayre (1978), who recorded about 15 individuals per groups in Malaysia; and four to eight individuals in coastal Sabah (Macdonald & Mason 1987).

Overall, the results of potential habitat observations and interviews indicate that the population of *A. cinereus* in Central Java is still relatively high. It is

Table 1 Prediction of the potential habitat of *Aonyx cinereus* in Central Java

Location	Habitat information
Pantai Gempol Sewu, Rowosari, Kendal	The former nests found around river deltas and fish ponds. The soil substrate is loose so that it can be easily dug as a nest hole. *A. cinereus* hunts the fish at the river. So, they build a nest not far from the river.
Petung river, Limpung, Batang	Small-clawed Otter built nests on riverbanks covered with shrubs and rocks.
Kemuning river, Batang	There is a fishpond that A. cinereus often visit. We found traces of *A. cinereus* on the soil substrate around the river (Figure 5a). The rivers and cornfields also can be potential habitats for *A. cinereus*
Tributary of Petung river, Limpung, Batang	We found feces in the edge of the river (Figure 5b). It is a fact that *A. cinereus* prefers to build the nest around the river near a fishpond to make it easier to find the food source.
Lampir river, Sangubanyu village, Bawang, Batang	Habitat is in a rocky river with not too heavy water flow. The river is bordered by agricultural land. we found about seven former nest spots
River near the coffee plantation, Bawang, Batang	Potential habitat is in rocky rivers. We found 2 (two) nests of *A. cinereus* between the rock crevices (Figure 5c). We also encountered fresh animal feces (Figure 5d)

Figure 5 Field sign of *A. cinereus*. (A) Traces of *A. cinereus* on the soil substrate around the Kemuning river, Batang; (B) *A. cinereus* feces in Tributary of Petung river, Limpung, Batang; (C) nests of *A. cinereus* in river near the coffee plantation, Bawang, Batang; (D) *A. cinereus* feces in river near the coffee plantation, Bawang, Batang

supported by the fact that the population frequently encounters in rice fields, rivers, fish ponds, ponds, and around the coast in several areas in Semarang, Batang, and Kendal - Central Java.

Bacterial Isolation and Identification

Nine swab samples were obtained and subjected for bacterial isolation and resulted in nine dominant colonies. The 16S rRNA sequences and Basic Local Alignment Search Tool (BLAST) analysis shows that the isolates belonged to *Citrobacter freundii* (n=3), *Klebsiella pneumoniae* and *Proteus alimentorum* (n=2), as presented in Table 2.

Potential Bacterial Zoonoses

This research is the first study to assess potential pathogens from *A. cinereus* in Indonesia to the best of our knowledge. In comparison, pathogenic bacteria were also studied in several otter species, including sea otters (*Enhydra lutris nereis*) in the USA (Miller *et al*., 2009) and Eurasian otter (*Lutra lutra*) in Portugal (Oliveira *et al*., 2008). Clostridium, Campylobacter and Vibrio were among bacteria that commonly detected in fecal samples. In our study, *Citrobacter freundi* and *Klebsiella pneumoniae* were among potential pathogens identified, which belong

Table 2 Bacterial isolates and identification results from nine swab samples

Isolate ID	Origin	Species	Identity (%)	Reference strain
B3	skin	*Citrobacter freundii*	99.77	ATCC 8090
B11	feces	*Citrobacter freundii*	99.88	ATCC 8090
B13	skin	*Citrobacter freundii*	99.88	ATCC 8090
B6	skin	*Klebsiella pneumoniae*	99.88	DSM 30104
B9	feces	*Proteus alimentorum*	99.53	DSM 104685
B2	anus	-	-	-
B10	feces	*Proteus alimentorum*	99.41	DSM 104686
B4	skin	-	-	-
B12	mouth	-	-	-

to the Enterobacteriaceae family that linked to human diseases. *Citrobacter freundi* can cause severe gastroenteritis, haemolytic uraemic syndrome (HUS), and urinary tract infections (Wanger *et al*., 2017; Tschape *et al.*, 1995). *Klebsiella pneumoniae* is a common cause of pyogenic hepatic abscess (Lederman & Crum, 2005). A comprehensive study is needed to assess more individuals of otter species in Indonesia. This study is based on preliminary research, but we strengthen the hypothesize that *A. cinereus* may pose a risk of spreading enteric pathogens to humans.

This study suggests that illegal trade needs monitoring to reduce harvesting activities in the wild and the biological risk of capturing Small-clawed Otter. Further ecological studies are required to examine how population trend and risk faced by Small-clawed Otter in Java as fundamental information for efficient wildlife management.

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