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Cellular immune responses and phagocytic activity of fishes exposed to pollution of volcano mud

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ABSTRACT

Since May 29, 2006, a mud volcano in the Brantas Delta of the Sidoarjo district has emitted mud that has inundated nearby villages. Pollution in this area has been implicated in detrimental effects on fish health. In fishes, leukocyte and phagocytic cells play a vital role in body defenses. We report for the first time the effect of "LUSI" volcano mud on the immune systems of fish in the Brantas Delta. The aim of this study was to find biomarkers to allow the evaluation of the effects of volcanic mud and anthropogenic pollution on fish health in the Brantas Delta. The study took places at the Brantas Delta, which was polluted by volcano mud, and at reference sites in Karangkates and Pasuruan. Leukocyte numbers were determined using a Neubauer hemocytometer and a light microscope. Differential leukocyte counts were determined using blood smears stained with May Grunwald-Giemsa, providing neutrophil, lymphocyte and monocyte counts. Macrophages were taken from fish kidney, and their phagocytic activity was measured. *In vitro* analyses revealed that leukocyte and differential leukocyte counts (DLC) were higher in *Channa striata* and *Chanos chanos* caught from the polluted area. Macrophage numbers were higher in *Oreochromis mossambicus* than in the other species, indicating that this species is more sensitive to pollution. In areas close to volcanic mud eruption, all specimens had lower phagocytic activity. Our results show that immune cells were changed and phagocytic activity was reduced in the polluted area indicating cytotoxicity and alteration of the innate immune system in fishes exposed to LUSI volcano mud and anthropogenic pollution.

1. Introduction

Fish blood is sensitive to pollution-induced stress, and changes in hematological and metabolic parameters can be used as toxicity indices of xenobiotics (Sancho et al., 2000). Blood indices are considered pathophysiological parameters of the whole body and therefore are important in diagnosing the structural and functional status of fish exposed to xenobiotics (Adhikari et al., 2004). In fish, cellular immune responses are important for immune defense; it is the fish's blood cells that hold the key to understanding the effects of pollutant exposure. Fish blood has a direct relation with immune system function and humoral defense in fish.

In fish, humoral defense plays a fundamental role in innate immune responses. The immune system of fishes consists of hematological cells that play an essential role in internal defenses. Leukocytes are responsible for internal defense in the immune system. Many leukocytes infiltrate infected areas to provide rapid protection. Differential leukocytes that play roles in fish immunity are neutrophils, lymphocytes, and monocytes. Neutrophils exhibit phagocytic activity and are capable of killing dangerous agents that enter the body. Lymphocytes are the most common circulating leukocyte in fish. Monocytes are large leukocytes with an abundant blue-gray cytoplasm that lacks granules and are occasionally vacuolated.

The immune defense mechanisms of fish seem to be related and similarly competent compared to those of mammals (Enane et al., 1993). As in mammals, monocytes migrate to tissues and become macrophages (Campbell and Murru, 1990). Macrophage count can be used to compare macrophage activity in fish from

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polluted and non-polluted rivers. In general, fish possess a well-developed, non-specific innate immune system, and phagocytes play an important role in fish defense (Secombes and Fletcher, 1992; Jensch-Junior et al., 2006). Phagocytic function has been used as an immunological parameter to evaluate the health status and immune function of different fish species subject to diverse biotic and a-biotic factors such as pollutants (Weeks and Warinner, 1986) and pathogens (Ainsworth and Dexiang, 1990). Many experimental and epidemiological reports have confirmed the immunotoxicity of pollutants in a variety of marine biota. However, the immune response, as measured in number of hematological cells, varies among species. For example, Shao et al. (2004) found that leukocyte numbers of normal fish are 20,000–150,000 cells/ml, while Johni et al. (2003) reported that total leukocyte concentrations of the grouper *Epinephelus fuscoguttatus* are 41,000–57,000 cells/ml. Studies of fish captured in sites polluted by sewage or exposed to other types of stressors showed that the numbers of hematological cells can change drastically, which influences fish immunity (Cazenave et al., 2005; Zutshi et al., 2010).

This study used *Oreochromis mossambicus* (local name: Mujaer), *Channa striata* (local name: Gabus) and *Chanos chanos* (local name: Bandeng) as model fish species. The first species is omnivorous, while the second species is carnivorous and has an additional respiratory organ or primitive labyrinth-like gills. The third species is herbivorous and is the most economically important fish of East Java. All species inhabit and are distributed widely at the Brantas Delta, which ranges from freshwater to saltwater. Our study was conducted at the Brantas Delta that includes the Aloo River and Sidoarjo brackishwater ponds close to the LUSI volcano mud. The delta contains several villages and serves as a source of economic activity for local people; in particular, the water is used for irrigation in agriculture, and the brackishwater ponds are used for aquaculture. Many brackishwater ponds (called "*tambak*") in East Java were built along the coast line for shrimp and milkfish aquaculture. Fishery production in brackishwater ponds in East Java plays an important role in supporting economic growth for this province. Waste from rice paddies and domestic settlement discharges into the Aloo river. Since May 29, 2006, an eruption of the 'LUSI' mud volcano has altered the physical and chemical conditions of the nearby wetland, particularly at the Brantas Delta. The LUSI mud has caused over 40,000 people to be displaced from more than a dozen villages in the area. The inundation of factories, farmlands and milkfish ponds has had a significant economic impact on the region. Overviews of the LUSI site and a history of eruptions have been reported by Mazzini et al. (2007), Cyranoski (2007a,b) and Davies et al. (2008, 2011). However, no published papers have reported the effect of LUSI volcano mud on fish. It is important that analysis be undertaken, not only to examine the mechanisms that triggered volcano mud, but also to identify impacts on the aquatic environment, including fishes that inhabit the nearby area.

Although the hematoxicity and immunotoxicity of fish are well established, studies of biological aspects, including immune responses of fishes exposed to volcano mud are scarce. Our previous works (Risjani et al., 1998, 2012; Risjani, 2007) showed that fish populations at the Brantas Delta decreased, so it is important to examine aspects of fish health, including immune function. The present study examined the influence of anthropogenic and volcano mud pollution at the Brantas Delta on immunity-related cellular responses of fishes, particularly the leukocyte, macrophage numbers and phagocytic activity. Our data could be used as baseline data for fish immune function, and they provide indicators of fish health during periods of toxic stress.

2. Materials and methods

2.1. Study area

The Brantas is the longest river in East Java, flowing 320 km from the spring river at Arjuno Mount to river mouths at the Strait of Madura. It flows through the following cities, which define the prominent zones: Malang, Blitar, Tulungagung, Kediri, Mojokerto and Surabaya (Fig 1). It drains an area of over 11,000 km^2 in East Java. LUSI is situated in the Sidoarjo district, close to the Porong River at the lower end of the Brantas. Much of the mud has been diverted to a nearby river and canal (Porong and Aloo), where it has formed a new 83-ha island and extended the natural delta.

Fish samples were taken from four sites (Fig 1) consisting of two investigated sites in the Brantas Delta influenced by LUSI mudflow and two other reference sites outside the delta:

a. The Aloo River located at Sidoarjo Regency – the Brantas Delta in East Java is 20 km long and has been characterized as a habitat for *O. mossambicus* and *C. striata*. This site is polluted by domestic, industrial and agricultural wastes and has been influenced by volcanic mud since 2006.
b. Brackishwater ponds for milkfish *C. chanos* culture situated at Sidoarjo – the Brantas Delta. These ponds are supplied by water from the Aloo River which is influenced by anthropogenic pollution and LUSI mudflow.
c. Karangkates Dam in an upstream part of the Brantas and is the reference site for *O. mossambicus* and *C. striata*.
d. Brackishwater ponds situated at Pasuruan were used as the reference sites for milkfish *C. chanos*.

The chemical-physical characteristics of the studied areas are listed in Table 1, providing several water quality parameters, including dissolved oxygen (DO), pH, salinity, TSS total suspended solid (TSS), chemical oxygen demand (COD) and Phenol. The Aloo River and brackishwater ponds of Sidoarjo that were influenced by LUSI volcano mudflow are characterized by a relatively higher TSS and COD and a lower amount of dissolved oxygen (DO). The phenol concentration is relatively higher only at the Aloo River. Salinity detected at the Aloo River was initiated by sediments input from LUSI volcano mud.

2.2. Animal collection

The aquatic animals used in this study were *O. mossambicus* (Cichlidae), *C. striata* (Channidae) and *C. chanos* (Channidae). *O. mossambicus* is commonly known as Java Tilapia and was discovered by Pak Mujaer in 1939 in East Java. Members of the local population call the fish *Mujaer*. The fish is omnivorous and tolerates a wide range of salinity, from fresh to salt water (euryhaline). *C. striata* is also called a snakehead fish, with the local name of *Gabus*. It has an additional respiratory organ or labyrinth-like primitive organ. The feeding habit is carnivorous. *C. chanos* is herbivorous and is known as the milkfish or *Bandeng* by local people. At each study site, five individuals of each species (thirty adult fish in total) with total lengths between 13 and 15 cm for *O. mossambicus*, between 18 and 25 cm for *C. striata*, and between 21 and 27 cm for *C. chanos* were collected.

2.3. Preparation of slides for hematology

Fish were anesthetized before blood sampling. Blood samples were taken from the caudal linea lateralis using a 22-G needle attached to a sterile plastic 1-ml syringe containing 50 µl of TBS