

# Isolation and identification of fungal infections causing death in leopard gecko's (*Eublepharis macularius*) eggs

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## ABSTRACT

Ten samples of Leopard gecko eggs contaminated by fungus were obtained from Pet Network Indonesia and several hobbyists in Surabaya using a purposive sampling technique. The sample selection were performed by looking at the presence of fungus attached to the egg shell indicates yellowish or reddish characteristics, bluish spots, and the presence of mycelium in the egg shell. Swabs were taken by swab technique and planted on natural media which was incubated at 25 °C for 4 days. The technique to identify the fungus were carried out using sticky tape taped to the surface of the colony which is then affixed to glass objects that have been given Lactophenol Cotton Blue staining. The data obtained were descriptively analyzed. The results indicate that from isolation and identification of fungus in leopard geckos' (*Eublepharis macularius*) egg samples contains *Fusarium* sp., *Cephalosporium* sp., *Rhizopus* sp., and *Aspergillus* sp. on the surface of the dead eggshell. In sum, Geckos' eggs have infected by several fungus causing death.

**Key words :** Leopard Gecko (*Eublepharis macularius*), Fungal-infection, Egg mortality, Embryo mortality.

## Introduction

Leopard gecko (*Eublepharis macularius*) is considered as one of the most common and popular animals in the reptile industry today. Leopard Gecko is one type of reptile that has a calm character, has a pungent odor compared to other reptiles, and does not need special attention. As reptile fans increase, more and more people are interested in becoming breeders, including the Leopard gecko breeder. In one mating female Leopard Gecko is able to store male sperm in its body and is able to lay eggs as much as 2 to 5 times a period (Farm innovator, 2015), each period of female eggs lay eggs as much as 1-2 eggs (Morgan, 2013)

Fungus cause losses due to failure of hatching

during egg incubation. Therefore it is necessary to do the isolation and identification of molds in Leopard gecko eggs to determine the type of mold that is pathogenic to Leopard gecko eggs, it is hoped that by knowing these pathogenic fungus, further research can be done to prevent fungal infection.

## Materials and Methods

Isolation and identification of fungus was conducted at Laboratorium of Bacteriology and Micology, Faculty of Veterinary Medicine Universitas Airlangga. Ten unhatched Leopard gecko eggs were collected from Pet Network Indonesia and pet lover from Surabaya through purposive sampling technique. Samples were collected from unhatched

eggs that showed characteristic such as yellowish and reddish, blue spot and the presence of micellium on egg shell. Fungus was collected using swab then inoculated on SDA using streak method then incubated on 25 °C for 4 days.

Fungus purification was conducted by transfer part of the fungus colony to new media (Alexopoulos, 1996). Fungus was inoculated using ose through streak method on SDA. It was incubated on 25 °C for 3-4 days then observed under microscope. Fungus was taken using transparent sticky tape then stick the tape on object glass which is dropped by Lactophenol Cotton Blue (Sastrahidayat, 2011). Hifa, spora and konidia form were observed under microscope using 100x, 400x, 1000x magnification.

**Results and Discussion**

It was observed there were 4 kinds of colony can be identified from the sample. They were colony of *Aspergillus* sp (Figure 1a), *Fusarium* sp. (Figure 1b), *Rhizopus* (Figure 1c) and *Cephalosporium* (Figure 1d) respectively.

Isolated colonies were observed under microscope using sticky tape method and Lactophenol Cotton Blue. Each colonies can be observed as below (Figure 2). It is suggested that the fail of hatch-

ing of Leopard gecko eggs was caused by fungus contained in the soil. Isolation and identification of the egg shell showed the dominance of *Fusarium* and *Aspergillus* (50%). The other fungus capable to infect the eggs of Leopard gecko are *Cephalosporium* (30%) and *Rhizopus* (20%). The eggs also can be infected by all those fungus in same time. *Fusarium* and *Aspergillus* are fungus colonies frequently causing death on reptile eggs (Herman, 2011) (Table 2).

*Fusarium* sp. Was found on the unhatched reptile eggs and cause mortality on the embryo (Moreira, 2005; Phillott, Parmenter, 2006; Praja, Yudhana, 2018). *Fusarium solani* significantly reduce the amount calcium on outer layer of egg shell. Fungus can extract the calcium compound from the egg shell caused deficiency on embryo. The penetration of its hifa is getting easier. This condition disturbs gas exchange, attacj the embryo, and/or delaying the maturation of embryo. It makes the shell thinner. Fungal infection can cause early hatching on Iberian rock lizard infected by fungus (3 days earlier than normal period). It decreases the level of survivability (Sastrahidayat, 2011).

*Cephalosporium curtipes* var. *uredinicola* was found infect unhatched egg of Loggerhead turtle (Dawson, 2011). Debyser and Zwart isolated *Cephalosporium* from whitemuscle lesion from *C. crocodilus* and lungs from kaiman (Huchzermeyer, 2003). *Rhizo-*

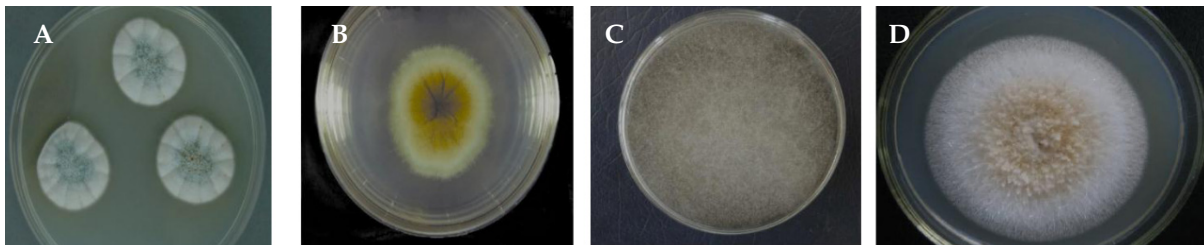


Fig. 1. There were 4 kinds of colony can be isolated from the samples. *Aspergillus* sp (A), *Fusarium* sp. (B), *Rhizopus* (C) and *Cephalosporium* (D)

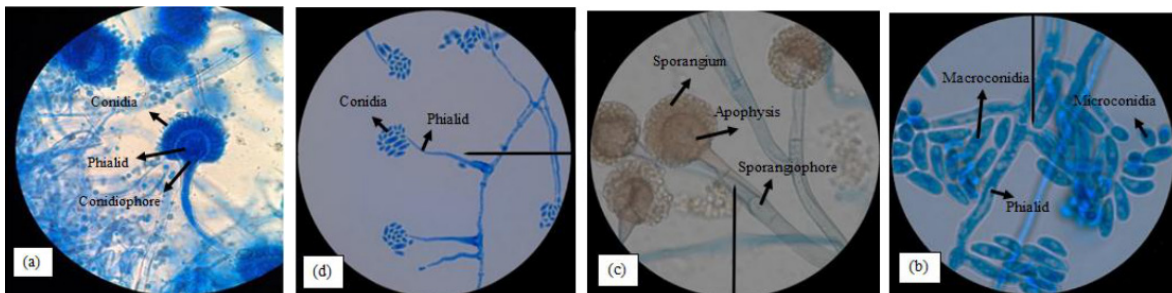


Fig. 2. Observation under microscope with 400x magnification. Each colonies can be distinguished as (a) *Aspergillus* sp., (b) *Fusarium* sp., (c) *Rhizopus* sp., *Cephalosporium* sp.

**Table 1.** Fungus identification on egg shell of Leopard gecko (*Eublepharis macularius*).

Method	Sample	Identification	
Isolation was conducted on Sabouraud Dextrose Agar; Identification was conducted using Lactophenol Cotton Blue	1	Yellowish spot on the shell, the presence of cotton like formation on the shell	<i>Fusarium sp.</i> <i>Aspergillus sp.</i>
	2	White cotton like formation on the egg shell	<i>Cephalosporium sp.</i>
	3	Yellowish spot on the shell, blueish spot is presence	<i>Fusarium sp.</i> <i>Rhizopus sp.</i>
	4	White cotton like formation on the shell	<i>Aspergillus sp.</i> <i>Cephalosporium sp.</i>
	5	White cotton like formation on the shell, blueish spot	<i>Rhizopus sp.</i>
	6	White cotton like formation on the shell	<i>Aspergillus sp.</i>
	7	Yellowish spot on the shell	<i>Chepalosporium sp.</i>
	8	Yellowish spot on the shell	<i>Fusarium sp.</i> <i>Aspergillus sp.</i>
	9	Yellowish spot on the shell, the presence of cotton like formation on the shell	<i>Fusarium sp.</i>
	10	Yellowish spot on the shell, the presence of cotton like formation on the shell	<i>Fusarium sp.</i> <i>Aspergillus sp.</i>

pus sp. is pathogen for amphibi, bird, fish, mamalia, and reptile. It can cause erosion or ulcer on skin. It is an opportunist pathogen and very dangerous for low antibody level host (Dawson, 2011). Reptile egg use egg shell as the first protection and as an environment for embryo development (Huchzermeyer, 2003). In this research *Rhizopus sp.*, *Fusarium sp.*, and *Aspergillus sp.* infected the samples decreasing the calcium level of the egg shell of the reptile (Herman, 2011). This condition rises the possibility of *Rhizopus sp.*, infection.

Infection of *Aspergillus sp.*, was suggested originally from the soil on the nest. *Aspergillus sp.*, can live well on 20-30 °C with humidity level 90% (Pratiwi, 2014). These conditions are suitable environment for incubating Leopard gecko eggs (Keller, 2017). The failure of hatching and mortality of embryo could be caused by some factor such as early hatching caused by oxygen level or decrease of water potency. Hatched embryo is smaller than normal but the survivability in wild is low (Solomon, 1980); decrease amount of calcium on the egg shell can disturb the normal condition of embryo development

**Table 2.** Percentage of fungus unhatched Leopard gecko eggs (*Eublepharis macularius*).

Fungi Species	Identified sample	Infection rate
<i>Fusarium sp.</i>	5	50%
<i>Aspergillus sp.</i>	5	50%
<i>Rhizopus sp.</i>	2	20%
<i>Cephalosporium sp.</i>	3	30%

(Herman, 2011; Keller, 2017); the disturbance of gas exchange caused by hifa penetration on egg shell (Pratiwi, 2014).

## Conclusion

In this research fungus can be isolated from unhatched Leopard gecko (*Eublepharis macularius*) egg shell are *Fusarium sp.*, *Cephalosporium sp.*, *Rhizopus sp.*, and *Aspergillus sp.* Infecting fungus have high percentage are *Fusarium* and *Aspergillus* (50%) then followed by *Cephalosporium* (30%) and *Rhizopus* (20%). According to the result all those fungus can infect the shell egg in a same time.

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