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Oxyuris spp. Infection on Green Iguana (*Iguana iguana*) Under Different Cage Types

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ABSTRACT

The caging system becomes one of the most crucial points in the green iguana (*Iguana iguana*) husbandry practice since some pathogens are transmitted through ingesting contaminated feed or water in the cage. One of the most common health problems in the green iguana is caused by infection of the gastrointestinal nematode (GIN), *Oxyuris* spp. Here, we conducted a study to identify *Oxyuris* spp. infection in the green iguana under different cage types in Malang Raya, East Java. A total of 40 fecal samples were collected and examined through flotation and modified McMaster techniques from three cage types, namely loose cage / extensive (n= 7), terrarium (n= 13), and iron-based cage (n= 20). The results showed that all green iguanas are infected with *Oxyuris* spp. (40/40 or 100 % prevalence). The highest mean egg per gram (EPG) was found in the green iguana which kept in a loose cage / extensive (mean EPG [CI 95 %] = 14 799.21 [14 442.05 – 15 156.37]), followed by terrarium (mean EPG [CI 95 %] = 8 763.80 [8 436.30 – 9 091.30]), and iron-based cage (mean EPG [CI 95 %] = 1 433.42 [1 303.79 – 1 563.05], respectively. At the same time, there is a significant relationship ($P<0.05$) between the type of cage and the infection rate of *Oxyuris* spp. According to this result, we recommend an iron-based cage with routine daily cleaning as prevention for *Oxyuris* spp. infection among the green iguana.

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INTRODUCTION

Reptiles are poikilothermic animals that have scaly and dry skin. The dry skin of reptiles functions to store body heat and control fluids that come out of the body. The mode of reproduction in most reptiles is oviparous by internal fertilization. The entire physiology and immune system of reptiles depend on temperature, which adjusts from sunrise to sunset in their natural habitat. Some types of reptiles that are often kept as pets include turtles, snakes, and *Iguana iguana*. Lizards have the highest number of species, approximately 4450 species with different types of habitats (Ekici *et al*., 2011; Zwart, 2022). Little is known about the diseases in green iguanas in Indonesia, but previous studies reported the infection of the gastrointestinal nematode, namely *Oxyuris* spp. (Oktaviana *et al*., 2019).

Indonesia is home to thousands of faunas, including 753 species of reptiles (Munir *et al*., 2019). The exotic addicted community is one of the popular communities in Malang, Indonesia, that love captive iguana as new companion animals in the household. *Iguana iguana* behavior is relatively easy to control, so owners only need to pay attention to good maintenance management and a comfortable cage environment (Tynes, 2010). Several types of *Iguana iguana* cages are often used by owners in Indonesia, including terrarium, loose (extensive),

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and iron cages. The base of the terrarium cage is made of cement or acrylic, with walls made of glass or acrylic. This type of cage has deficiencies in the circulation system and cleanliness of the cage base because it allows *Iguana iguana* feces to contaminate the feed. This allows for a high rate of endoparasite infection. These included the oocysts of Isospora and Eimeria, as well as the eggs of the Pharyngodonidae family (Nematoda; Oxyurida), Ascarididae, and Rhabditoidea (Nematoda), and Trematoda (Rom *et al.*, 2018). The loose cage type has a base in the form of soil with walls in cement or iron wire. The circulation system of the loose cage type is quite good but has deficiencies in the cleanliness of the bottom of the cage because it allows the feed to be contaminated with feces and soil. This allows for a high rate of endoparasite infection. This type of iron cage consists of a base and walls in the form of perforated iron so that the circulation system is perfect, and when the *Iguana iguana* defecates, the feces will not remain on the base of the cage. This can minimize the possibility of endoparasite infection. Feed that is not cleaned beforehand can be a source of transmission of endoparasites (Dowling, 2008).

Several nematode species, such as *Oxyuris* spp., *Capillaria* spp., *Strongyloides* spp., and cestode species, such as *Mesocstoides* sp., can infect *Iguana iguana*. The most common nematode species found in the digestive system of *Iguana iguana* is *Oxyuris* spp. (Ekici *et al.,* 2011). Infection occurs when *Iguana iguana* ingests worm eggs of *Oxyuris* spp. in the L3 phase infective via contaminated feed or water. The larvae will go to the small intestine and move to the large intestine, which then migrate to the cecum mucosa, develop into the adult stage, and settle in the lumen (Taylor, 2016). Ekici *et al.* (2011) state that severe infection will show clinical symptoms such as significant weight loss, diarrhea, anorexia, and regurgitation.

According to research done in Brazil by Teles *et al.* (2017), 66.6% of iguanas were infected with nematodes. Oxyurida nematodes were the most prevalent, with a high average intensity. Massive infections from iguanas can result in cloacal prolapse, gastrointestinal system blockages, and even mild localized inflammation. Green iguanas are economically significant since they have grown to be one of the most popular non-traditional pets, necessitating this research. Malang Raya has not seen the conduct of a study of this kind. It would help the community of people who are addicted to exotics and raise the standard of Iguana upkeep management. The purpose of this study was to ascertain the prevalence of *Oxyuris* spp. infection in green iguana housed in various types of cages in Malang Raya, East Java.

MATERIALS AND METHODS

Ethical Statement

All procedures of this study were approved by the Institutional Animal Care and Use Committee of Brawijaya University, Malang, East Java, Indonesia (Approval Number: 071-KEP-UB-2022).

Fecal Samples Collection

Fecal sample collection was carried out simultaneously during dry season from 11 December 2022 – 20 April 2023. A total of 40 fecal samples of *Iguana iguana* were taken from seven owners which reflect seven difference sub-districts (Sukun, Dau, Junjero, Batu, Bumiaji, Gedangan, and Lowokwaru) in Malang Raya Region, East Java, Indonesia (Figure 1).

Three grams of fecal samples were taken from each green iguanas, placed in plastic bag (contain formalin 10%), labelled and distributed to the Laboratory of Helminthology, Brawijaya University, Malang. The sampled green iguana were categorized into this following age group, i.e. juvenile (0-12 months), pre-adult (12-36 month) and adult (6-8 years). All fecal samples were collected in the early morning during basking and feeding time.

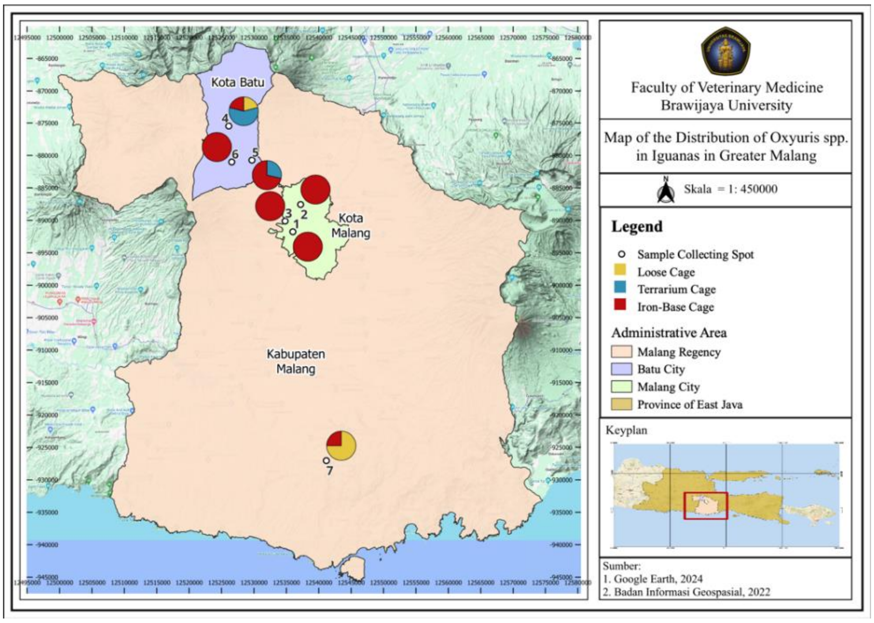

Figure 1. The study area in the Malang Raya Region was determined from seven owners, which reflect seven different sub-districts suitability circled in white.

Environment and Management Assessment

Assessment for environmental and management factors was done through direct inspection at the time of the sampling period. The cage's temperature and humidity were measured using Weather weather-free applications (Google, United States of America). Furthermore, we divided the cage type into three categories, namely (A) loose cage, a free-ranging room cage, the structure was made by a concrete wall which has direct contact to the soil as bedding. According to its size, the loose cage was measured as 300 × 200 × 250 cm and 150 × 90 × 120 cm. The cage was supplemented with branches mimicking the nest in the natural habitat of the green iguana; (B) terrarium, a glass cage with a sheet of newspaper as bedding material, and supplemented with shelter or branches; and (C) iron cage, a whole cage made from iron bars (Figure 2).

Questioner

The questionnaire is provided with open questions that target the iguana's owner to identify the husbandry practice or management system. The questions include feeding system, caging system, and health caging management.

Coprological Techniques and Microscopic Examination

Techniques for fecal sample examination used native, flotation and modified McMaster (Paramitha *et al.*, 2019). The *Oxyuris* spp. egg was morphologically identified and count under a light microscope. Egg count indicates the infection degree, and it states as egg per gram of fecal sample (epg) (Zajac *et al.*, 2021). Measurement of the length and width of the *Oxyuris* spp. egg for morphometrical purposes was done by Optilab Advanced Plus Camera® (Miconos Corp., Yogyakarta, Indonesia) and ImageJ® bundled with Zulu OpenJDK 13.0.6 application (Koukalova and Medvedova, 2016).

Data Analysis

A descriptive analysis was conducted on the examination results of the feces. According to Slusarewicz *et al.* (2019), the degree of infection was categorized concurrently by the level of nematode endoparasite infection: mild infection (201–500 EPG), medium infection (501–1000 EPG), and high infection (>1001 EPG). The association with different types of cages on the infection rate of *Oxyuris* spp. on Iguana iguana was analyzed using the Statistical Package for the Social Sciences (SPSS) application with a chi-square test followed by a likelihood-ratio alternative test.

Figure 2. The green iguana's cage; A). A loose cage / extensive; B). Terrarium cage; C). Iron-base cage

RESULTS AND DISCUSSION

Environment and Management Aspects

According to the direct assessment of management system, we found that seven green iguanas placed into loose cage, 13 green iguanas in an individual terrarium and 20 green iguanas in an individual iron cages. Although the assessment confirmed the constant temperature (22 – 32 ºC) in all study areas, but the humidity level are more varies. The humidity level of Sukun, Lowokwaru and Dau were 40 – 50 %, while Bumiaji, Junjero, Batu and Gedangan found to be more humid (50 – 70 %).

Fecal Samples Examination Results

Iguana iguana is one of the most popular types of lizards to be used as pets. The coprological techniques confirmed a hundred percent prevalence of *Oxyuris* spp infection in the green iguanas (100 %). *Oxyuris* spp. is one of the helminth resides in colon of *Iguana iguana*. Holland *et al.* (2008) confirmed the presence of *Oxyuris* spp. in decending colon of *Iguana iguana* through ultrasonography. However, infected animals did not show any of clinical symphtoms and animal seems to be healthy.

Oxyuris spp. infection in the green iguana also found in some previous studies in Turkey (Ekici *et al.*, 2011) and Banyuwangi, East Java, Indonesia (Oktaviana *et al.*, 2019). Infection is related to some behavioral factors, such as licking the cage or objects in the cage so the eggs are ingested by the green iguana, and ingestion of contaminated feed by eggs or infective larvae. According to (Barten 2003), unwashed feed or improper washing could transmit endoparasites to the green iguana.

By this study showed measure the morphologically features of *Oxyuris* spp. egg which represented in Figure 3. The egg is asymmetrically oval with a thin transparent wall containing embryos. The egg size ranged between 55.1 – 68.3 µm in length and 26.4-33.1 µm width. (Oktaviana *et al.*, 2019) mentioned that the size of *Oxyuris* spp. egg in the green iguana is 57 µm × 31 µm which completed with an operculum and blunt on the reverse site. The morphology of *Oxyuris* spp. egg is similar to *Pharyngodon* spp. eggs, but those two could be differentiate by the structure of the walls and the size of the eggs. The *Oxyuris* spp. egg has monolayer wall structure, while *Pharyngodon* spp. covered by the bilayer wall. Additionally, the *Pharyngodon* spp. egg is larger (92 – 104 µm × 58 – 67 µm) than in *Oxyuris* spp. (Rosyida *et al.*, 2023). According to (Oktaviana *et al.*, 2019), the parasite induced mild to moderate symptoms on the host, such as diarrhea, weakness, anorexia, and weight loss. However, fatal occurrences of green iguana occurred in heavily infected animals and co-infection with other gastrointestinal parasites and microorganisms. Serious intestinal lesions were caused by this, and sepsis could result from them (Raś-Noryńska and Sokol 2015; Arabkhazaeli *et al.*, 2018).

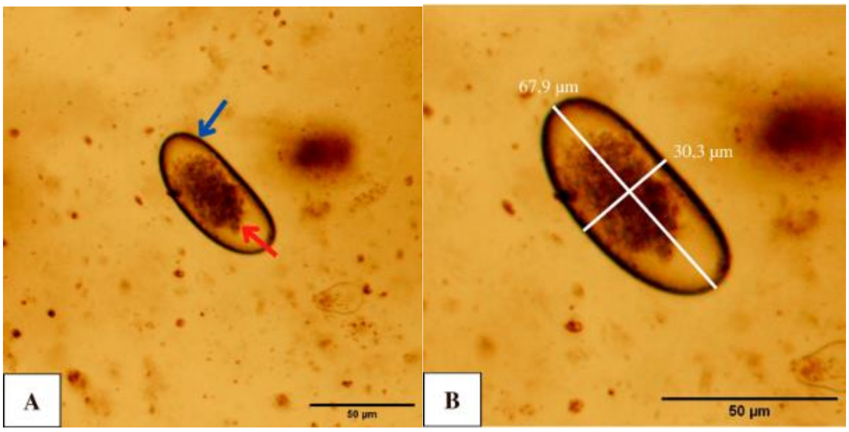

Figure 3. Egg morphology and morphometry of *Oxyuris* spp. in *Iguana iguana* in Malang Raya. (A) blue arrow implies the egg shell, while blue arrow is embryos; (B) Egg size measurement.

Table 1. Prevalence and mean EPG of *Oxyuris* spp. in *Iguana iguana* under different type cages

Type of cages (number of animals [n])	n Positive (% prevalence)	Mean EPG (CI 95 %)*
Loose cage / extensive (n= 7)	7 (100 %)	14 799.21 (14 442.05 – 15 156.37)
Terrarium (n= 13)	13 (100 %)	8 763.80 (8 436.30 – 9 091.30)
Iron-based cage (n= 20)	20 (100 %)	1 433.42 (1 303.79 – 1 563.05)

**CI= Confidence interval (CI) 95 %*

Table 2. The relationship between type of cages of *Iguana iguana* and the infections rate of *Oxyuris* spp**.**

Types of Cages	Infection Rate of *Oxyuris spp.* on *Iguana iguana*		P-Value
	Medium	**High**	
Loose cage / extensive (n= 7)	-	7 (100%)	0.000
Terrarium (n= 13)	13	-	
Iron-based cage (n= 20)	20	-	
Total Samples	**33 (82.5%)**	**7 (17.5%)**	

*n = number of samples; P = prevalence.

The modified McMaster technique confirmed the infection degree of *Oxyuris* spp. in green iguana in each cage type (Table 1). Based on the likelihood ratio test, the Asymp value was obtained. Sig. (2-sided) 0.000, which can be seen in Table 2. Asymp value. Sig. (2-sided) <0.05 indicates that there is a relationship between the type of rearing cage (terrarium, loose, and iron) and the infection rate of *Oxyuris* spp. on *Iguana iguana* in Malang Raya. The highest infection degree found in the green iguana kept under loose cage or free ranged room cage (mean EPG [CI 95 %] = 14 799.21 [14 442.05 – 15 156.37]), followed by terrarium (mean EPG [CI 95 %] = 8 763.80 [8 436.30 – 9 091.30]), and iron-based cage (mean EPG [CI 95 %] = 1 433.42 [1 303.79 – 1 563.05]. Although iguanas enabled to express the natural behavior such as walking and climbing on the additional substrates, such as branch of tree, but as a naturally solitary reptile, the communal cage might induce the tension and stress to them. Increasing of stress level of the host is in line with the increasing of host susceptibility to parasite infection as well as the host mortality due to parasite (Lafferty and Kuris, 1999). Furthermore, Dowling *et al.* (2008) mentioned that the high population density of *Iguana iguana* in the cage could be a risk factor for high infection rates of *Oxyuris* spp.

The high prevalence and infection degree of *Oxyuris* spp. might also related to the cleanliness program. We found that the owner conducted a once-a-week cleaning program by sweeping the soil floor without any disinfection. As the soil-base cage, the loose cage found to be more humid and fecal heap are prominent in some spots. Perfect humidity provides suitable environment for parasite development especially in tropical countries, including Indonesia. Perry and Moens (2011) said that the nematode eggs can persist in dry conditions or environments such or adhered in dry objects surrounding the fecal spots as soil or substrates in the loose cage.

The green iguanas on another two cage types (terrariums and iron-based cage) are moderately-infected with *Oxyuris* spp. We identified two types of terrariums. The first terrarium was 300 × 150 × 200 cm in size, placed outdoor, with a dense population of 11 adult *Iguana iguana.* The second one is indoor terrarium sized 40 × 30 × 30 cm and became a place for two juvenile *Iguana iguana*. *Oxyuris* infection among green iguanas in terrarium cages are closely related to the trapped fecal materials which caused direct contaminated to animals. Since the cage was categorized into communal cage with the high density, (Hedley *et al.* 2013) mentioned that overcrowded can lead to higher endoparasite infection rates. Even though a daily cleaning by brushing and water spraying was done in the morning, it could not prevent reinfection since there is no soap or disinfectant added. Cleaning the cage by spraying and brushing without soap enabled the *Oxyuris* egg still stick to the bottom of the cage, although in relatively small amounts (Barten, 2003).

The lowest infection intensity found in the green iguana kept under individual iron-based cage size of 60 × 40 × 40 cm. According to Hatfield (2004), iron cages have a good pedestal system because the feces will fall directly past the base of the cage. In this case, the iron-based cage is equipped with a mat under the cage so the leftover and fecal materials are easy to clean and prevent fecal contamination to the iguana's feeding. The cage is also provided with substrates, i.e., tree branches or hanging tools, so that fecal contamination to feed can be avoided. Moreover, the cage is cleaned once a day by brushing and water-spraying at the rail to prevent the fecal matter from getting stuck to the iron of the cage.

CONCLUSION

The green iguana housed in a loose cage or extensive had the highest mean egg per gram (EPG) following terrariums, and iron-based cages were the next highest EPGs. Based on our research, we firmly believe that the ideal option for the green iguana is a single, iron-based cage. This enclosure effectively expresses the green iguana's natural behavior and prevents *Oxyuris* spp. infection. On the other hand, it must be done with better anthelminthic therapy and a cleaning regimen, such as daily cage and food cleaning.

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