UNDERGRADUATE THESIS

MOLECULAR IDENTIFICATION OF *Ascaridia columbae* IN DOMESTIC PIGEON (*Columba livia domestica)* USING POLYMERASE CHAIN REACTION (PCR) IN SURABAYA, JAWA TIMUR

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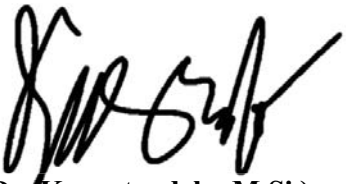
Approval of
Supervising Committee,



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DECLARATION

Hereby, I declared that in this thesis entitled:

MOLECULAR IDENTIFICATION OF *Ascaridia columbae* IN DOMESTIC PIGEON (*Columba livia domestica*) USING POLYMERASE CHAIN REACTION (PCR) IN SURABAYA, JAWA TIMUR

There is no other work ever published to obtain a college degree in certain college and to my knowledge there is also no work or opinion ever written or published by others, except those in writing referred to this paper and mentioned in the references.

Surabaya, 29 September 2022

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SUMMARY

THERESIA MONIKA. This research is entitled "Molecular Identification of *Ascaridia columbae* in Pigeons (*Columba livia domestica*) Using Polymerase Chain Reaction (PCR) in Surabaya, East Java" under the supervision of Dr. Kusnoto, drh., M.Si. as the first supervisor and Prof. Dr. Wurlina, drh., M.S. as the co-supervisor.

Ascaridia columbae (*A. columbae*) is a roundworm from the nematode class and family Ascaridiidae with the genus Ascaridia that infects specific hosts, namely birds of the Aves class. This nematode typically infects the small intestine of the host, where it can inhibit the host's productivity and causes ascaridiasis.

Identification of this parasite is generally carried out by examining morphological characteristics in the adult worm stage, larvae stage, and egg stage (Ibrahim *et al*., 2018). A more accurate diagnosis is made possible by using molecular identification, which can also supports the diagnosis made by morphological observations. This technique can also be the foundation for phylogenetic research of a parasite species (Chaudhary *et al*., 2017).

Pigeons which have high mobilization capability have the potential to be a reservoir of parasites that can transmit disease to other birds. Helminthiasis, an infection of worm endoparasites in pigeons, is one of the illnesses that typically affects them and has still not been paid much attention by breeders. One of these helminthiasis diseases is ascaridiasis.

Considering that study on the molecular identification of *A. columbae* species in Indonesia has never been conducted, a study about molecular

identification of *A. columbae* using the Polymerase Chain Reaction method is required in order to acquire more precise identification result (Ashfiyah *et al*., 2022) and (Muqorobin *et al*., 2017). To detect the strain of this species and differetiate it from other nematode species, the primer utilized in this work is a specific primer, namely Perlprimer which was created based on the Cox1 mtDNA gene area held by *A. columbae*. Molecular identification based on DNA sequences is one of the fundamentals in determining the molecular taxonomy of species in order to validate a nematode species and avoid misdiagnoses.

This research is an exploratory laboratory study that aims to identify the resemblance of DNA band length (bp) of mtDNA Cox 1 gene region from *Asacridia columbae* isolated from pigeons in Surabaya, East Java with the DNA band target of an *A. columbae* specific primer from Karbala Province by using the polymerase chain reaction (PCR) technique. This research result can be used as a basis for molecularly identifying the nematode *A. columbae* in order to ensuring that the results of this identification are more precise and can prevent misdiagnosis of the parasite in the city of Surabaya, East Java. The samples used in this study were isolated and identified from 68 sets of small intestines of domestic pigeons obtained from traditional markets in Surabaya. The isolated samples were then identified by morphological observations both macroscopically and microscopically before being sent for molecular identification examination using the PCR method.

According to research findings, out of a total sample of 68 sets of small intestines of domestic pigeons, 30 *A. columbae* worms were discovered in 5 samples of small intestine that were positively infected. From 34 sets of pigeon

small intestine samples from Wonokromo Traditional Market, 5 slaughter pigeon small intestine samples were tested positive for *A. columbae* infection with a prevalence of 14.7%. While results from 34 sets of pigeon small intestine samples from Darmo Permai Traditional Market showed that all pigeon small intestine samples were tested ngative for *A. columbae* infection with a prevalence of 0%. Thus, the prevalence of *A. columbae* infection in Surabaya was 7.36%.

The results of the morphological examination revealed that *A. columbae* have microscopic morphology with unique characteristics on the anterior in the form of mouth formation with triradiate lips equipped with cephalic alae on the lateral part of the body; while on the posterior side, the formation of spicules, annulated cuticle, anus, and precloacal sucker can be seen. The macroscopic morphology findings revealed that *A. columbae* have length of 18-35 mm with a cylindrical body shape and yellowish-white color.

Five of the 30 samples of *A. columbae* were coded A. col and then DNA was extracted using a Qiagen Qiamp mini kit, yielding 60 μL of DNA template. This template DNA was then amplified using a specific primer for the Cox 1 mtDNA region. The specific primers were PerlPrimer Forward which had a length of 22 nucleotide bases and PerlPrimer Reverse with a length of 20 nucleotide bases with a target DNA band at 263 bp fragment.

The temperatures used in the PCR method in this study were 94°C for 5 minutes for the pre-denaturation procedure, followed by denaturation at 94°C for 30 seconds, annealing for 1 minute at 52°C, extension at 72°C for 30 seconds. 3

minutes, and the final extension at 72°C for 5 minutes which was repeated for 35 cycles.

The results of DNA amplification which have been visualized in agarose gel 2% are mtDNA Cox 1 amplification seen as a single DNA band with a 263 bp fragment.

Because this research still has to be further examined using sequencing techniques and phylogenetic tree analysis, the molecular identification of the *A. columbae* species in this study has not been entirely accurate.

MOLECULAR IDENTIFICATION OF *Ascaridia columbae* IN DOMESTIC PIGEON (*Columba livia domestica*) USING POLYMERASE CHAIN REACTION (PCR) IN SURABAYA, JAWA TIMUR

Theresia Monika

ABSTRACT

Ascaridia columbae is an endoparasite commonly found in the small instestine of domestic pigeons and is considered as a common occurance in the poultry business. This current study was conducted to determine the molecular identification of *A. columbae* using Polymerase Chain Reaction (PCR) methods. 30 samples of *A. columbae* were collected from 68 domestic pigeon's small intestine found in Surabaya. Five samples of *A. columbae* were subjected to undergo molecular identity analysis using PCR and were coded as A.col. The other 25 samples were subjected to undergo permanent preparations and staining using Semichen-Acetic Carmine for morphological observation. The prevalence of *A. columbae* infection obtained in this study was 7.36%. Spesific primer sequence of mtDNA Cox 1 for *A.columbae* were used in this study to amplificated the sample's DNA template. The result obtained were the DNA amplification of the sample (A.col) and supported by the morphological examination of the sample. The PCR product A.col under UV transluminator showed a single DNA band at 263 bp fragment which is in align with the specific primer. This research aims to molecularly identified *A. columbae* spesies found in Surabaya which could provide a more precise identification data of these parasite spesies found in Surabaya, East Java.

Keywords: *Ascaridia columbae*, domestic pigeons, molecular identification, PCR.

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Surabaya, 29 September 2022

Author

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ABBREVIATIONS AND SYMBOLS

PCR	: Polymerase Chain Reaction
DNA	: Deoxyribonucleic acid
rRNA	: Ribosomal ribonucleic Acid
bp	: Base Pairs
Mg2+	: Ion magnesium
K+	: Ion potasium
rDNA	: Recombinant Deoxyribonucleic acid
RNA	: Ribonucleid acid
spp	: Spesies
kb	: Kilo Base Pairs
cDNA	: Complementary Deoxyribonucleic Acid
rDNA	: Ribosomal Deoxyribonucleic Acid
mtDNA	: Mitochondrial Deoxyribonucleic Acid
dNTP	: Deoxynucleoside triphosphate
dATP	: Deoxyadenosine triphosphate
dCTP	: Deoxycytidine triphosphate
dGTP	: Deoxyguanosine triphosphate
dTTP	: Thymidine triphosphate
°C	: Celcius
COX1	: Cytochrome C Oxidase 1
NaCL	: Natrium Chloride
ATL	: Tissue lysis buffer
AL	: Lysis buffer
AW1	: Wash buffer 1
AE	: Elution buffer
AW2	: Wash buffer 2
TBE	: Tris/Borate/EDTA
UV	: Ultraviolet