CHAPTER 1 INTRODUCTION

1.1 Background

Ascaridia columbae (*A. columbae*) is a roundworm belongs to the nematode class and family Ascaridiidae with the genus Ascaridia (Mehlhorn, 2016). 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). In Indonesia, the identification method is generally carried out by staining permanent preparations with Semichen-Acetic Carmine as well as by examining worm eggs in poultry feces (Ashfiyah *et al*., 2022) and (Muqorobin *et al*., 2017). However, this examination requires specialized abilities, particularly in fixation and staining processes, therefore a lack of such knowledge can result to parasitic misdiagnosis and incorrect medication (Poon *et al*., 2018).

Molecular identification provides a more precise diagnosis which can also supports the diagnosis made by morphological observations and this method can be used as the foundation for phylogenetic studies of a parasite species (Chaudhary *et al*., 2017). Salem *et al*. (2022) stated that Ascaridia spesies are known to be able to infect pigeon particularly *A. columbae* and *A. galli*. These two species are known to have similar morphological ultra structures with slight differences in the teeth section (Ibrahim *et al*., 2018). Molecular identification can also strengthen diagnosis results based on morphological observations, especially in distinguishing *A. columbae* and *A. galli* species found in pigeons. A study on the molecular identification of *A. columbae* using the PCR method is required in order to obtain more precise identification results especially because the research on *A. columbae*

that has been conducted in Indonesia is still only based on morphological identification (Ashfiyah *et al*., 2022) and (Muqorobin *et al*., 2017).

This nematode of the genus Ascaridia is known to only infect specific hosts, namely birds that belong to the Aves class (Al Quraishy *et al*., 2020). These nematodes typically affect the digestive tract and can inhibit the productivity of their host (Hamzah *et al*., 2020). Salem *et al*. (2022) also stated that *A. columbae* can infect pigeons of all ages and this infection could be fatal in young pigeons under the age of 12 weeks.

A. columbae (Gmelin, 1790) is known as the only enteric worm from the genus Ascaridia that infects pigeons and can cause ascaridiasis in both high and low populations (Bessat and Dewair, 2019). This disease can result in weakness from anemia, diarrhoea, weight loss, digestive tract issues, and even death (Mehlhorn, 2016). Additionally, this disease also causes a decrease in egg productivity and egg quality (Mubarokah *et al*., 2019). The pathogenicity of the worms from the Ascaridia genus in causing harm to and obstruction of the intestinal tract in the host's body are the cause of the direct losses experienced by breeders (Sharma *et al*., 2019).

These endoparasites are generally localized in the digestive tract of poultry and can survive in the digestive tract of their hosts for up to one year (Bizhga *et al*., 2011). The main source of transmission of this endoparasite is through the oral route by consuming feed contaminated with worm eggs where the second stage infective larvae of this endoparasite develop in the eggs (Mehlhorn, 2016). The adult worm of *Ascaridia sp*. can produce up to thousands of eggs each day, and the eggs of this

species are known to be highly resistant to environmental conditions and are able to persist in the infective stage for months to years in the environments where animals and humans live (Bessat and Dewair, 2019). Transmission from endoparasites is also supported by the role of intermediate hosts such as grasshoppers and earthworms (Ibrahim *et al*., 2018).

Both domesticated and wild pigeons are vertebrates from the winged Aves class with good light ability and location recall capability (Kadri *et al*., 2016). Pigeons as an animals with high mobilization capabilities, especially for foraging purposes and habitat purposes have the potential to be a reservoir of parasites that might transmit diseases to other birds especially to an endangered bird species and consumption poultry species (Salam *et al*., 2018).

Pigeons are one of the many birds that are widely kept by the residents of Surabaya City as a hobby and consumption purpose as well as for bird racing (Muqorobin *et al*., 2017). According to the Surabaya City Livestock Office in 2020, the population of pigeons in Surabaya City have reached 4,999 heads. Pigeons are also known as livestock that are easy to care for and are very easy to be cultivate so that their potential as livestock is quite high (Kadri *et al*., 2016). Apart from being a hobby, pigeons in Indonesia are also bred for human consumption purpose (Priosoeryanto *et al*., 2005).

Nevertheless, Indonesian pigeon husbandry is known for its low productivity (Muqorobin *et al*., 2017). Like other poultry farms, this low level of productivity is is brought on by variables related to livestock health, namely pigeon illnesses (Priosoeryanto *et al*., 2005). One of the diseases that generally infect

pigeons is helminthiasis, which is an infection of worm endoparasites in pigeons and has still not been given much attention by breeders (Muqorobin *et al*., 2017). One of the helminthiasis diseases suffered by pigeons is ascaridiasis caused by *A. columbae*. Accurate identification of *A. columbae* species in pigeons can prevent medication errors due to misdiagnosis of the parasite.

In this study, *A. columbae* worms were molecularly identified using the PCR (Polymerase Chain Reaction) technique with a specific primer designed by Hamzah *et al*. (2020). Molecular identification based on DNA sequences is one fundamental methods in determining the molecular taxonomy of species and validating the nematode species (Singh *et al*., 2015). The PCR technique has the benefit of allowing for the precise detection of parasite DNA in the nanogram and picogram range, which helps to avoid incorrect diagnoses (Bazh, 2013). 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 Perprimer which was created based on the Cox1 mtDNA gene area held by *A. columbae*. Considering that research on the molecular identification of *A. columbae* species in Indonesia has never been conducted, understanding the molecular profile of this nematode is required to improve the accuracy of identification of *A. columbae* species and can be used as an important reference in further research related to this species in Indonesia.

This research purpose is to determine the molecular profile in the form of DNA band length (bp) from *A. columbae* isolated from pigeons in Surabaya City by analyzing DNA bands from the mtDNA region Cox 1 in accordance to the DNA band target of a specific primer designed by Hamzah *et al*. (2020).

1.2 Problem Formulation

Based on the background, the formulation of the problem that can be proposed:

1) Does the nematode *Ascaridia columbae* isolated in the small intestinal tract of pigeons in Surabaya, East Java have a DNA band length which matches the DNA band target from specific primer of *A. columbae* in the mtDNA Cox 1 gene region by using polymerase chain reaction technique?
2) What is the prevalence of *A. columbae* infection isolated in the small intestinal tract of pigeon in Surabaya, East Java obtained in this research?

1.3 Theoretical Basis

The nematode known as *A. columbae* have a cylindrical body shape, are semi-transparent white with a protruding mouth surrounded by triradiate lips, and have cephalic alae that stretch from the lateral ide to the filariform esophagus (Salem *et al*., 2022). According to Mehlhorn (2016), this worm has two sexes, each with a different body size. Male worms have two identically sized spicules and a precloacal sucker with chitinous edges (Salem *et al*., 2022). While female worms have the capacity to deposit eggs with smooth surfaces and thick walls that are embryo-free (Mehlhorn, 2016). Ascaridiasis or *A. columbae* worm infection, can lead to digestive problems in the definitive host. While widespread infections can result in mild catarrhal enteritis and intestinal obstruction, moderate helminth infections can be linked to mucosal enteritis (Salam *et al*., 2018).

Molecular identification of *A. columbae* was conducted to improve the accuracy of *A. columbae* species identification and to provide precise information

about its phylogenetic relationship within the Ascaridiidae family. One of the techniques used for molecular identification of *A. columbae* is Polymerase Chain Reaction.

In identifying molecular parasites, PCR has the benefit of being able to detect specific parasitic DNA ranging from nanograms to picograms in order to identify molecular parasites and avoid misdiagnosis (Bazh, 2013). These benefits also result to high accuracy in nematoda identification in both the egg and adult stages, which is crucial for genetic studies as well as elements of parasite diagnosis, parasite prevention, and disease control (Sim *et al*., 2010).

In order to detect diseases, identify genetics, detect fingerprints, conduct paternity tests, and do DNA computing, the PCR technique allows short DNA fragments to be amplified many times exponentially up to 10 kb (Rahman *et al*., 2013). Molecular identification of *A. columbae* using the PCR method was carried out by testing and analyzing the mtDNA Cox 1 gene region, which serves as a molecular marker to assess interspecies phylogenetic and differential identification of nematode within the order Ascaridida (Kalyanasundaram *et al*., 2017). According to Sadaow *et al*. (2018), (Chaudhary *et al*., 2017), and (Poon *et al*., 2018); Polymorphic markers, such as mitochondrial cytochrome C oxidase subunit 1 (COX 1), are frequently utilized in molecular epidemiology research specifically to identify nematode species. Because this gene enables differentiation between closely related worm species, the mtDNA Cox 1 gene sequence is useful for resolving nematode phylogenetic relationship (Singh *et al*., 2015). This gene region has the potential to serve as a genetic marker in the nematode phylum and is

frequently utilized in identifying parasitic species and the evolutionary relationships between parasites (Kalyanasundaram *et al*., 2017).

The PCR test's molecular identity findings are represented by DNA band lengths (bp) that *A. columbae* owns in the mtDNA Cox 1 gene region. The DNA band length (bp) that has been discovered can be utilized to molecularly identify this species and as a starting point for further research regarding the evolutionary relationship of these species in the genus Ascaridia as well as a basis to learn more about the connections between the species discovered in other countries. In the research conducted by Hamzah *et al*. (2020) regarding the molecular profile of the mtDNA Cox 1 gene region from *A. columbae* in the province of Karbala, it was found that the DNA band length was 263 bp fragment.

The essential elements for each PCR test are DNA templates, primers, nucleotides, and DNA polymerases (Garibyan and Avahia, 2013). In addition there are supporting components in each PCR test in the form of buffer compounds, sterile water, and additional reagents containing Magnesium salt Mg2+, Potassium salt K+, dimethylsulfoxide, formamide, bovine serum albumin, and Betaine (Lorenz, 2012). This procedure involves a DNA extraction process, denaturation of the double-stranded genomic DNA template, annealing, and an extension process (Gupta, 2019 and Tavares *et al*., 2011). These three processes were carried out at different temperatures, namely denaturation of the DNA template at 94°C, annealing at 52°C, and the extension process at 72°C (Gupta, 2019).

1.4 Aim of Research

The aims of this research are:

1) Identifying 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.
2) Knowing the prevalence of *A. columbae* infection isolated in the small intestinal tract of pigeon in Surabaya, East Java.

1.5 Outcome of Research

The outcome of this research are anticipated to offer details about the resemblance of DNA band (bp) of the mtNDA Cox 1 gene region from *Ascaridia columbae* isolated from pigeons in Surabaya with the DNA band target of an *A. columbae* specific primer which 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. Additionally, it is anticipated that the information from this study could be used as a reference for further study on the phylogenetic analysis of the nematode species *A. columbae* in Indonesia, particularly in the city of Surabaya, East Java.