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# Molecular Identification of *Ascaridia galli* in Chickens from Traditional Markets in Surabaya, Indonesia

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## ABSTRACT

**Background:** *Ascaridia galli* is pathogenic in chickens and causes substantially reduced growth rates, weight loss, low egg production and death in infected chickens. It is necessary to carry out research on the molecular identification of *A. galli* in chickens in traditional markets in Surabaya using the Polymerase Chain Reaction (PCR) method with the COX-1 mtDNA gene primer.

**Methods:** This research was carried out from April to July 2023. The number of samples used in this research was 100 chicken small intestines taken from traditional markets in Surabaya, Indonesia. *A. galli* worms obtained from worm collections were identified according to the morphology of the *A. galli* worms. DNA extraction was carried out followed by PCR laboratory procedure. The specific primers utilized were designed to target the COX-1 gene and the amplified products were visualized in 1.5% agarose.

**Result:** The *A. galli* worm has a long body posture. Male *A. galli* worms measure 30-50 mm long; while, adult female *A. galli* worms measure 65-80 mm long. The results of electrophoresis with a 2% agarose gel run for 30 minutes at 100 Volts and visualized under ultraviolet light using a UV Transilluminator (Geldoc) showed the presence of a single band of the COX-1 mtDNA gene in the 533 base pair. Infection with *A. galli* worms often causes reduced growth rates and decreased body weight, which is associated with increased worm weight.

**Key words:** *A. galli*, Chickens, COX-1, Infectious disease, PCR.

## INTRODUCTION

Ascariasis is an infectious disease caused by the parasitic worm *Ascaridia galli* (Zalizar *et al.,* 2021). This worm is a parasitic nematode that is often found in poultry, including chickens (Sharma *et al.,* 2019; Tu *et al.,* 2016). This parasite infection occurs when birds swallow eggs containing larvae in food or drink (Shohana *et al.,* 2023). One of the factors for *A. galli* infection in chicken is the traditional rearing system, in which the chickens are free to roam and easily get infected (Setyowati *et al.,* 2022). This parasite is pathogenic in chickens and causes substantially reduced growth rates, weight loss, low egg production and death in infected chickens. Therefore, it is necessary to follow an effective parasite control and prevention program (Biswas *et al.,* 2021).

The presence of *A. galli* worms is still a major obstacle in the poultry industry and can cause economic losses (Alrubaie, 2015). Infection in chickens is diagnosed by detecting eggs in fecal examination (Manjunatha *et al.,* 2023). Accurate identification is important to prepare treatment and prevention strategies for this nematode infection (Roeber *et al.,* 2013). Specific and more accurate methods need to be used to identify various species, especially the *A. galli* species, one of which is through DNA analysis (Bazh, 2013). The latest progress in genetic analysis based on DNA is using mitochondrial DNA which is suitable for analysis at the species level and has been carried out on various gene targets.

*A. galli* infection can trigger an inflammatory reaction in the small intestine of its definitive host. Another impact

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that occurs is chronic bleeding due to migrating larvae causing gastrointestinal damage including gastritis, enteritis and ulceration of the digestive tract (Ritu *et al.,* 2023). The severity of damage to the intestinal mucosa depends on the number of worms in the intestine. Any mucosal inflammation is generally followed by digestive disorders, absorption of nutrients such as electrolytes, vitamins, mineralsand secretion of substances that play a role in the process of digestion of food (Barkas *et al.,* 2013). Worm infections also cause a reduction in food fluids and intestinal blockages by roundworms and tapeworms as well as lumps in the intestines (Mbanga *et al.,* 2019). The spread of endoparasites to chickens can occur through contaminated feed, water and livestock equipment (Nemathaga *et al.,* 2023).

Morphological examination is not as accurate as genetic analysis. Molecular techniques developed based on the study of genetic markers, especially the COX-1, ITS-1 and ITS-2 genes, are very specific for determining species differences in the genus Ascaridia (Chen *et al.,* 2022). The COX-1 gene is an mtDNA gene that codes for the synthesis of the Cytochrome C Oxidase subunit 1 protein (Pietan *et al.,* 2016). This gene can be used as a molecular marker to determine the genetic structure of populations of the *A. galli* species (Höglund *et al.,* 2012). So far, the molecular identification method that has often been used to determine the *A. galli* species is Polymerase Chain Reaction (PCR).

This method is often used because it has a high level of accuracy in product amplification and species determination, the microorganisms detected do not have to be alive and do not require a long time (Garibyan and Avashia, 2013).

Research by Shohana *et al.* (2023) shows that the percentage of *A. galli* worms in the small intestine of chickens is 66% in duodenum, 36% in jejunum and 26% in ileum. The high percentage of *A. galli* worm infection can be influenced by several factors; namely, season, maintenance, management and cage cleanliness (Zalizar *et al.,* 2021). Based on the background that has been described, this study was aimed at carrying out molecular identification of *A. galli* in chickens in traditional markets in Surabaya using the Polymerase Chain Reaction (PCR) method with the COX-1 mt DNA gene primer.

## MATERIALS AND METHODS

### Study area and sample collection

This research was carried out from April to July 2023. The number of samples used in this research was 100 chicken small intestines collected from traditional markets in Surabaya, Indonesia. Isolation of *A. galli* worms from samples was carried out at the Parasitology Laboratory, Airlangga University; while, molecular identification using PCR was carried out at the Tropical Disease Institute, Airlangga University.

### Morphological identification of *A. galli*

The small intestine that has been obtained was opened using a scalpel and surgical scissors at the end slowly. The contents of the intestine were removed, filtered using a sieveand observed for worm infestation. The collected wormes were placed on a petridish in physiological NaCl.

*A. galli* worms obtained from worm collections were counted and identified according to the morphology using Semichen Acetic Carmine staining. The stained worms were placed on an object glass, clamped with a cover glass and observed under a microscope with 100x magnification.

### Molecular identification of *A. galli*

DNA extraction was carried out following the PCR laboratory procedure. Five microliters of DNA was utilized as a template. The specific primers utilized were designed to target the COX-1 gene (Table 1). Mixture for the PCR reaction with a volume of 25 µl consisting of 12.5 µl PCR Mastermix (0.5 U Taq Polymerase, 0.2 mM dNTP, 1.5 mM MgCl2 and Buffer 1x), 1.25 µl of each primer and 5 µl DNA template.

The PCR was performed under the following conditions: initial denaturation at 94°C for 7 minutes, 35 cycles of 96°C for 50 seconds of denaturation, 50°C for 40 seconds of annealingand 72°C for 1 minute of extension and finally 10 minutes of extension at 72°C. The amplified products were visualized in 1.5% agarose gel in a UV transilluminator with a 360 nm wave length.

### Data analysis

The data obtained from this research was in the form of descriptives and the images of morphology and molecular analysis of the *A. galli* worm using Polymerase Chain Reaction (PCR).

## RESULTS AND DISCUSSION

The results of dissection of 100 small intestines in free-range chickens obtained from a Surabaya traditional market showed the presence of *A. galli* infection. Macroscopically, the *A. galli* worm had a large, long body of yellowish white color, which was found in the small intestine of infected chickens (Fig 1). The *A. galli* worm has a long body posture. Male *A. galli* worms measure 30 - 50 mm long, while adult female *A. galli* worms measure 65 - 80 mm long.

**Table 1:** Primer arrangement used in the PCR method.

| Species | Primer | Base pair | Reference |
|---|---|---|---|
| *COX-1* | F: 5′ - ATT ATT ACT GCT CAT GCT ATT TTG ATG-3′<br>R: 5′ - CAA AAC AAA TGT TGA TAA ATC AAAGG-3′ | 533 bp | (Shuai *et al.,* 2023) |

Note: F = Forward; R = Reverse.

Microscopic identification of *A. galli* worms was carried out using Semichen-Acetic Carmine staining and viewed with a binocular microscope at 100x magnification. At the beginning of microscopic identification, it can be seen that in the anterior part there is a mouth equipped with three large lips, one lip on the dorsal part and two on the lateroventral part, esophagus and cuticle. In the posterior, the male *A. galli* worm has a spicula, pre-cloacal sucker and anus. Observation of the morphology of the *A. galli* worm in the anterior part shows the cuticle, esophagus*and* mouth which is equipped with three lips (Fig 2).

*A. galli* was first discovered in Germany. Later, it was reported in Brazil, India, Zanzibar, the Philippines, the Belgian Congo (Democratic Republic of the Congo), China, Canada*and* the United Kingdom (Balqis *et al.,* 2017). *A. galli* is commonly found in the small intestine of the chickens, turkeys, geese, guinea fowl and many wild birds, but is most often found in chickens and has a widespread distribution throughout the world (Shifaw *et al.,* 2021; Shohana *et al.,* 2023). Adult worms can move through the lumina of the large intestine and cloaca and end up in the oviduct, where they can enter the chicken's egg (Salem *et al.,* 2022).

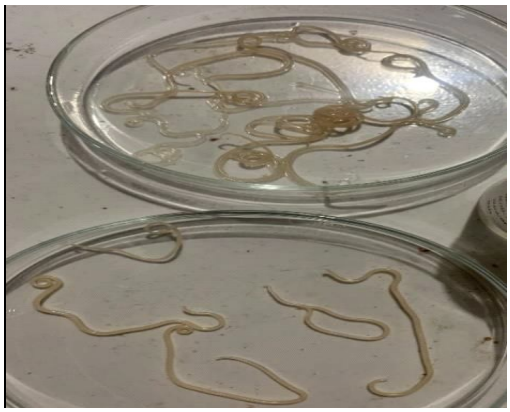
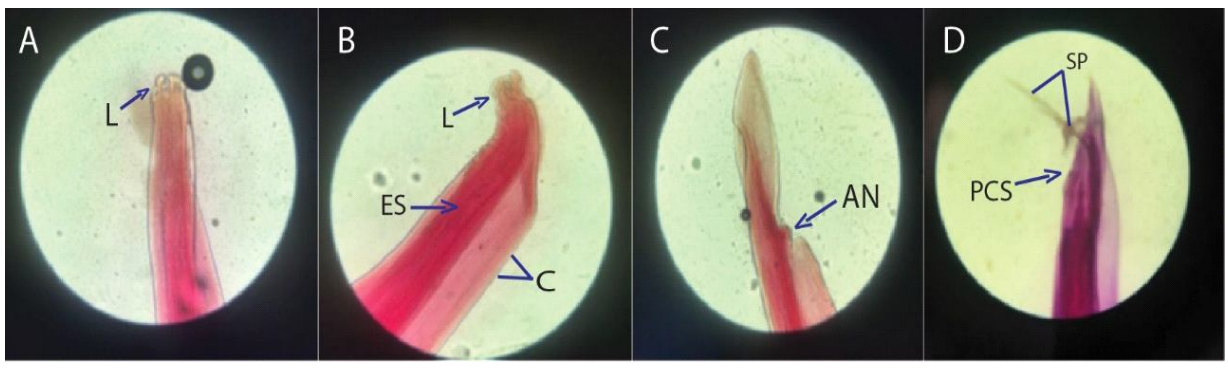


**Fig 1:** Collection of *A. galli* from the small intestine of chickens.

Under ideal laboratory conditions, almost 88% of *A. galli* eggs placed in host feces finish developing in about one to two weeks (Rahimian *et al.,* 2016). Infectious eggs build up in the environment because, while the majority of ascarid eggs are destroyed within a few months, up to 3% of them can survive for up to two years (Thapa *et al.,* 2017). As part of their development phase, the larvae emerge from the eggs after consumption and pierce the intestinal membrane (Ferdushy *et al.,* 2012). This results in irritation, inflammation and damage to the mucosa (Pleidrup *et al.,* 2014).

Some larvae quickly return in the intestinal lumen to mature into adult worms. After approximately five weeks, these worms attain sexual maturity and, if they are not removed, excrete the parasite's eggs for several months (Stehr *et al.,* 2018). A high worm burden is linked to appetite loss, diarrhea*and* mechanical intestinal blockage (Torres *et al.,* 2019). These symptoms can then cause a decrease in food absorption and the liver's ability to store fat (Sharma *et al.,* 2018). Additionally, it has been demonstrated that severe illnesses decrease weight growth and may even raise mortality (Hinrichsen *et al.,* 2016).

Molecular identification of *A. galli* worms using Polymerase chain reaction (PCR) by amplifying COX-1 gene was carried out in this study. The results of electrophoresis with a 2% agarose gel run for 30 minutes at 100 Volts and visualized under ultraviolet light using a UV Transilluminator (Geldoc) showed the presence of a single band of the COX-1 mtDNA gene of 533 base pairs (Fig 3). Polymerase Chain Reaction has more specific and accurate results in detecting disease infections molecularly (Yang and Rothman, 2004). Molecular techniques developed based on the study of genetic markers, especially the COX-1, ITS-1 and ITS-2 genes, are very good for determining the differences in species of the genus Ascaridia (Chen *et al.,* 2022). Similar to the results of present study, Mlondo *et al.* (2022) also amplified a




(A and B): C = Cuticle; ES = Esophagus; L = Mouth equipped with three lips. Posterior morphology of *A. galli* (C and D): AN = Anus; SP = Spicules; PCS = Pre-Cloacal Sucker.

**Fig 2:** Anterior morphology of *A. galli.*

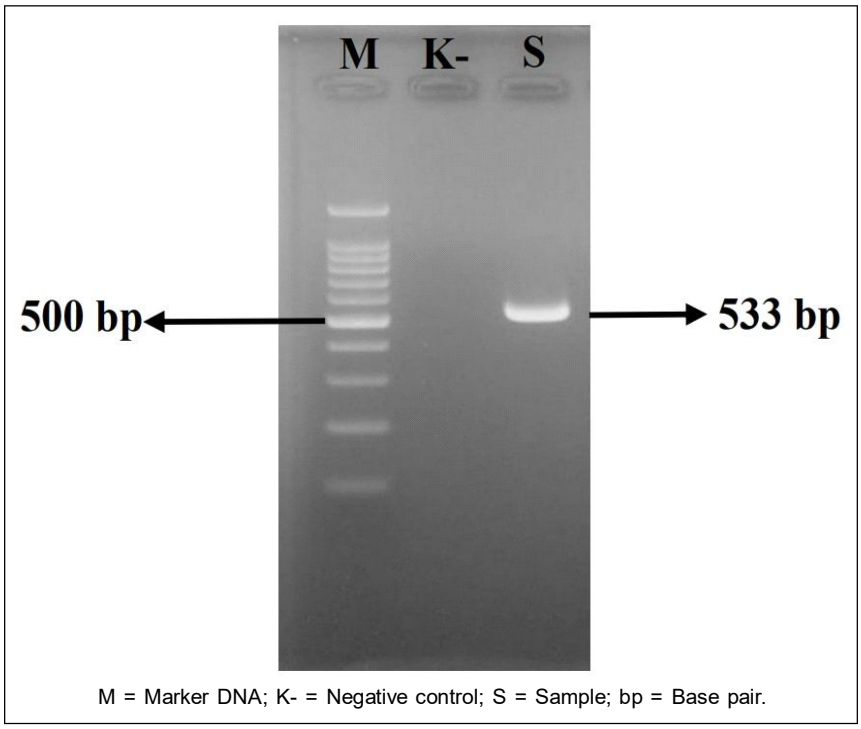



**Fig 3:** PCR products were visualized via electrophoresis using 2% agarose gel.

fragment of 533 base pairs of COX-1 gene of *A. galli*. Chen *et al.* (2022), targetted the ITS1 region of COX-1 primer and amplified a fragment of 655 bp. Biswas *et al.* (2021) also amplified the fragments of COX-1 gene using primers ITS1 and ITS2 as well as primers JB3 and JB4.5.

Applying an anthelmintic treatment to the birds is one of the best ways to manage ascariasis (Tarbiat *et al.,* 2016; Toor and Goel, 2022). This is occasionally significant because, despite the fact that infection with *A. galli* triggers particular immune responses, the protective effect is dubious (Sharma *et al.,* 2019). According to all available data, flocks of laying hens on litter typically carry some level of infection with them, which can occasionally even get worse as they get older and reach the conclusion of the production cycle, which can take up to 80 weeks (Zloch *et al.,* 2018). A number of medications have been demonstrated to be effective against *A. galli*, including benzimidazoles (fenbendazole and flubendazole), imidazothiazoles (levamisole and pyrantel) and macrocyclic lactones (ivermectin) (Soudkolaei *et al.,* 2021; Feyera *et al.,* 2022).

## CONCLUSION

The nematode worm *A. galli* infects the small intestine of free-range chickens, which was confirmed from the samples collected from the markets located in Surabaya traditional markets. Based on macroscopic, microscopic and molecular biology analysis of DNA using PCR with the COX-1 mtDNA target region sequence. The specific PCR amplification of a fragment of 533 bp size in this studyconfirmed the species.

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### Conflict of interest

The authors declare that there is no conflict of interest.

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