

IN VITRO DEVELOPMENT OF *Ascaridia galli* EGGS INTO INFECTIVE EGGS AND LARVAE OF STADIUM 2 (L2)

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ABSTRACT

The study aimed at finding out the development of *Ascaridia galli* (*A. galli*) eggs that were given aerator treatment and those without aerator treatment into infective eggs and L2 through *in vitro* culture. Each treatments has 108,000 eggs assigned to 8 groups of 1,000; 2,000; 3,000; 4,000; 5,000; 6,000; 7,000; and 8,000 eggs, respectively with 3 repetitions. Female *A. galli* were collected from the small intestinal lumen of naturally infected domestic chickens. The eggs collected from the uterus of adult female *A. galli* were incubated in sterile aquadest at ambient temperature for 45 days (without aerator) and 25 days (with aerator) to obtain the infective eggs and the L2. The number of the infective eggs and hatched L2 were counted under stereo microscope. Data were analysed descriptively. There were 97.740 eggs (90.5%) in the groups without aerator developed into infective eggs and 77.040 eggs (71.3%) developed into the L2. Meanwhile, there were 101.847 eggs (94.3%) in the groups with the aerator developed into the infective eggs and 88.722 eggs (82.15%) hatched L2. It is concluded that the eggs collected from worms uterus had high viability and the aerator application shortened the developing period of the *A. galli* worms.

Key words: *Ascaridia galli*, infective eggs, *in vitro*, larvae of stadium 2

ABSTRAK

Penelitian ini bertujuan mengetahui keberhasilan perkembangan telur menjadi telur infeksiif dan L2 *Ascaridia galli* (*A. galli*) melalui kultur *in vitro* antara yang diberi perlakuan aerator dan tidak. Masing-masing perlakuan menggunakan 108.000 telur yang dibagi dalam 8 kelompok dengan masing-masing kelompok terdiri dari 1.000, 2.000, 3.000, 4.000, 5.000, 6.000, 7.000 dan 8.000 telur cacing *A. galli* dengan 3 kali ulangan. Cacing *A. galli* betina diperoleh dari dalam lumen usus halus ayam kampung yang terinfeksi secara alami. Telur cacing yang diperoleh dari uterus cacing betina *A. galli* dewasa diinkubasi dalam aquadest steril pada temperatur kamar selama 45 hari (tanpa aerator) dan 25 hari (dengan aerator) untuk mendapatkan telur infeksiif dan L2. Jumlah telur infeksiif yang berkembang dan yang menetas menjadi L2 dihitung di bawah stereo mikroskop. Pada kelompok tanpa aerator, jumlah telur yang berkembang menjadi telur infeksiif adalah 97.740 telur (90,5%), dan yang berkembang menjadi L2 adalah 77.040 telur (71,3%), sedangkan pada kelompok dengan aerator, jumlah telur yang berkembang menjadi telur infeksiif adalah 101.847 telur (94,3%), dan yang berkembang menjadi L2 adalah 88.722 telur (82,15%). Dapat disimpulkan bahwa telur yang diambil dari uterus cacing memiliki viabilitas yang tinggi dan penggunaan aerator mempersingkat waktu perkembangan cacing *A. galli*.

Kata kunci: *Ascaridia galli*, telur infeksiif, *in vitro*, larva stadium 2

INTRODUCTION

Ascaridia galli (*A. galli*) is the most common type of parasitic nematodes found in chickens (Fahrimal and Rafflesia, 2002). *Ascaridia galli* has been reported in the small intestine of chicken, turkey, goose, duck and the others fowl (Susanti and Prabowo, 2014). The application of anthelmintics is one of common method to cope with ascariasis. It is known that extensive application of the anthelmintics to control worm infestation in animal husbandry is the main causal factor of resistance (Coles, 2005). Therefore, there have been many studies that made use of natural substances as alternative anthelmintics against *A. galli* (Tangalin, 2011; Tiwow *et al.*, 2013; Balqis *et al.*, 2017; Prastowo *et al.*, 2017).

The studies of the efficacy of natural substances as anthelmintics against *A. galli* were conducted both *in vitro* (Rahimian *et al.*, 2016; Tarbiat *et al.*, 2018) and *in vivo* (Prastowo *et al.*, 2017). The *in vitro* studies were conducted to adult worms, worms at larva stadium and worms at egg stadium. The eggs in the majority of the studies of *A. galli* infection were collected from worm uterus and there were only a few studies (Luna-Olivares *et al.*, 2012; Ferdushy *et al.*,

2013) that used the eggs isolated from feces. The majority of recent studies examine the supporting factors of experimental infection, including the selection of incubation media (Permin *et al.*, 1997; Kaingu *et al.*, 2013), incubation temperature and moisture (Onyirioha, 2011; Tarbiat *et al.*, 2015), egg's resistance to environmental condition (Katakam *et al.*, 2014), and the correlation between hospes and infection period (Idi *et al.*, 2004; Gauly *et al.*, 2005), genotype (Kaufmann *et al.*, 2011) and nutrient (Das *et al.*, 2011). Adult female worms laid only mature eggs (Kim *et al.*, 2012), and all of the eggs coming from uterus were immature or unable to complete embryonation process (Tiersch *et al.*, 2013). Therefore, it is necessary to conduct a study about the development of *Ascaridia galli* eggs that were given aerator treatment and those without aerator treatment into infective eggs and L2.

MATERIALS AND METHODS

The study was conducted in the Department of Parasitology of the Faculty of Veterinary of Gadjah Mada University, Yogyakarta, Indonesia. Worms were collected from the small intestine of naturally infected

chickens slaughtered the slaughtering house in Terban, Yogyakarta, Indonesia.

The Treatment of Female Adult *A. galli* Worms

The *A. galli* worms were put into beaker glass containing 0.62% NaCl. All of the worms were washed several times using the solution to completely remove impurity. Female worms were selected as indicated by their larger size and their straight posterior. Subsequently, the female worms were put into beaker glass containing 0.62% NaCl (Balqis *et al.*, 2009).

The Collection of *A. galli* Eggs

All of the collected worms were cut on their posterior porus genitalis that was bright-dark border and then the eggs were taken along with their uterus by squeezing the bodies of the worms to obtain 2 g eggs, then the bodied were disposed. The eggs of the worms were put into beaker glass containing 50 mL of 0.5 N NaOH. The eggs in the solution were then stirred using magnetic stirrer for 30 minutes. Subsequently, they were kept motionless to precipitate and the supernatant was removed. The process was repeated 3 times to ensure the eggs free from uterus debris (Prastowo and Ariyadi, 2015).

The solution containing the eggs was centrifuged 3 times and the eggs were ready for embryonization. The number of eggs obtained from a number of adult female *A. galli* was counted under microscope. The eggs were precipitated, mixed with 1 ml of aquadest and put into eppendorf tube. The egg content of 100 μ L of homogeneous egg suspension in solution was taken and counted in 3 repetitions. The number of eggs was determined by counting the number of the eggs from the population of dissected worms with following formula: 10x mean egg content of 100 μ L of the solution (Tiuria, 1991). The distribution of the number of eggs to 1,000; 2,000; 3,000; 4,000; 5,000; 6,000; 7,000; and 8,000 followed Balqis *et al.* (2009) with modifications. There were 8 groups with 3 repetitions.

The Development of the Infective Eggs and the L2 of *A. galli*

The eggs of the worms in the group without any aerator treatment were cultured *in vitro* by incubation in plastic Petri dish containing aquadest at ambient temperature for 45 days to let the eggs develop into infective eggs and L2. Meanwhile, the eggs in the group with aerator treatment were cultured *in vitro* incubating in Erlenmeyer tube containing aquadest at ambient temperature for 25 days to let the eggs to develop into infective eggs and L2. Aerator machine was fastened on the Erlenmeyer tube and turned on for 24 hours throughout the experiment (Prastowo and Ariyadi, 2015). The number of the eggs developed into infective eggs and L2 was counted under stereomicroscope.

Data Analysis

The mean of the eggs developed into the infective ones and the L2 in various groups were analyzed descriptively.

RESULTS AND DISCUSSION

There were 108,000 eggs used in the study which were assigned to the group without aerator and the group with aerator. The number of eggs in each solution (density) did not affect the hatchability (Table 1 and 2). The result was consistent with the finding of Balqis *et al.* (2009) in which the density did not affect hatchability and the development of infective eggs reached 89.4%.

The Eggs Development without Aerator

There was variation in the number of the eggs in the groups without aerator treatment that developed into the infective eggs (Table 1, Figures 1a and 1b). The total number of the eggs that developed into the infective eggs was 97,740. It showed that the capability of the eggs cultured *in vitro* without any aerator was high enough (90.5%). Meanwhile, the variation of the number of the eggs developed into L2 without aerator treatment was summarized in Table 1 and Figure 1c. It showed that the capability of the eggs cultured *in vitro* was high enough with 77,040 eggs developed into L2 (71.3%).

It was clearly observed in Table 1 that the developing capability of the eggs collected from the uterus of adult female worms was high enough. It was on the contrary to the results of the study by Tiersch *et al.* (2013) showing that all of the eggs collected from the uterus were not mature and unable to complete embryonization process. The results of the study were also higher than those of the study by Rahimian *et al.* (2016) showing that less than 40% of the eggs collected from the uterus were able to complete embryonization process. It was believed that the difference was caused by the difference in the determination of the uterus part that should be taken to obtain the eggs. The exactness in determining the uterus part to cut to collect the eggs influenced how many eggs would be successfully collected. Graybill (1924) suggested that the eggs in the posterior of the uterus could be considered as the ones that would be able to develop and perfectly hatch and their dimension was almost similar to the dimension of the ones found in feces.

The Development of the Eggs with Aerator

There was variation in the number of the eggs in the group with the aerator treatment that developed into the infective eggs (Table 2). The total number of the eggs that developed into the infective eggs that developed into the infective eggs was 101.847 with the percentage of 94.3% and the total number of the eggs that developed into the L2 was 88.722 (82.15%).

As the case of the development of the eggs without any aerator, it was clearly observed in Table 2 that the developing capability of the eggs cultured *in vitro* with the aerator was higher than the developing capability of the eggs cultured without any aerator treatment. The results of the study showed that the use of the aerator improved the developing capability of the eggs of the worms. The aerator was an instrument to move air that the air was rich of oxygen and its temperature was

Table 1. The development of eggs into infective eggs and L2 without aerator

Number of <i>Ascaridia galli</i> eggs	Infective eggs		L2	
	Average (\pm SD) number	Percentages (%)	Average (\pm SD)	Percentages (%)
1,000	880 \pm 34.641	88	720 \pm 60.000	72
2,000	1,800 \pm 60.000	90	1,420 \pm 34.641	71
3,000	2,700 \pm 60.000	90	2,220 \pm 60.000	74
4,000	3,660 \pm 60.000	91.5	2,860 \pm 124.900	71.5
5,000	4,500 \pm 60.000	90	3,480 \pm 180.000	69.6
6,000	5,400 \pm 60.000	90	4,140 \pm 261.534	69
7,000	6,420 \pm 120.000	91.7	720 \pm 60.000	72
8,000	7,220 \pm 242.487	90.2	1,420 \pm 34.641	71

Table 2. The development of eggs into infective eggs and L2 with aerator

Number of <i>Ascaridia galli</i> eggs	Infective eggs		L2	
	Average (\pm SD)	Percentages (%)	Average (\pm SD)	Percentages (%)
1,000	910 \pm 38.197	91	811 \pm 15.395	81
2,000	1,854 \pm 65.023	92.7	1,670 \pm 43.301	83.5
3,000	2,745 \pm 56.560	91.5	2460 \pm 8.660	82
4,000	3,740 \pm 161.093	93.5	3,260 \pm 376.431	81.5
5,000	4,710 \pm 174.940	94.2	4,215 \pm 300.541	84.3
6,000	5,628 \pm 140.310	93.8	4,824 \pm 153.727	80.4
7,000	6,762 \pm 516.578	96.6	5,775 \pm 584.615	82.5
8,000	7,600 \pm 343.948	95	6,560 \pm 580.000	82

**Figure 1.** The development of *Ascaridia galli* eggs. a= First stadium of *Ascaridia galli* eggs; b= *Ascaridia galli* infective eggs; c= *Ascaridia galli* L2.

well-maintained. The use of it influenced the survival of the eggs of the worms. Kim *et al.* (2012) suggested that the development of *Ascaris suum*'s eggs was highly influenced by temperature. On the contrary, routine change in the temperature was not favorable for the development of eggs that it decreased the viability of the eggs (Mero and Gazal, 2009). Additionally, the use of the aerator also shortened the developing period of the eggs. It was consistent with the opinion of Kim *et al.* (2012) suggesting that proper temperature would accelerate the development process of *A. suum*'s eggs.

The high percentage of the eggs that could develop into the infective eggs and the L2 was indicative of the high capability of the female *A. galli* worms in laying eggs and in good survival of the eggs in external environment so that it was important to comprehensively control of the infection of *A. galli* in chickens.

CONCLUSION

The eggs collected from worms uterus had high viability and the aerator application shortened the developing period of the *A. galli* worms.

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