



SURAT KETERANGAN

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Telah melaksanakan penelitian dengan judul sebagai berikut :

No	Judul Karya Ilmiah	Tahun Pelaksanaan Penelitian
1	Immunomodulatory Activity of Black Jinten Oil (<i>Nigella sativa</i>) as Macrophage Activator for <i>Salmonella typhimurium</i> Infected Rat	2020
2	Screening the Reproductive Tract of Dairy Cattle for Pathogenic Micros	2019
3	Human Chorionic Gonadotropin (hCG) from Urine of Pregnant Women to Manipulate in vivo Ovulation and Pregnancy of Madura Cows	2019
4	Anti Early Embryonic Protein (EEP) for Pregnancy Test by Microtiter Strip in Dairy Cows	2019
5	The Effect of Feeding High Level of Protein on Reproductive Performance of Bali Starling.	2019
6	Antisperm Antibody in Repeat Breeder Friesian Holstein Cows at KPSP Setia Kawan Nongkojajar, Tutur District, Pasuruan, Indonesia.	2019
7	Diagnosis of Single and Twin Pregnancy, and Early Embryo Mortality Through Progesterone Level Test on Local Does.	2019
8	Improvement of Pregnancy Rate in Bali Cows with the Combination of Equine Chorionic Gonadotropine (eCG) from Local Pregnant Mare with PGF _{2α} .	2019
9	Progesterone Profile of Dairy Cows which Experienced the Failure of Pregnancy to Artificial Insemination (AI).	2019
10	Effect of Heat Shock Protein (HSP) in Post Thaw Baluran Bull Semen	2018
11	Potency of Mycotoxin Binders on MDA Level, Expressions of Caspase 9 and Caspase 3 in The Uterus of Mice Exposed to Zearalenone	2017



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12	Polymorphism of Growth Hormone Gene in The Artificial Insemination Result of Madura Cattle with Limousin Semen as a Reference for Genetic Selection	2018
13	Implementation of fotogrametry techniques as body mass estimation of indo-pacific bottle nose dolphin (Tursiops aduncus) in bali dolphin lodge	2020
14	Uji Sensitivitas Kebuntingan Sapi Perah Menggunakan Pregnancy Specific Protein B (PSPB) Microtiter Strip dan Progesteron sebagai Gold Standard	2007
15	Estimation of Equine Chorionic Gonadotropin (eCG) concentrate in the Blood Sera of Pregnant Mare	2014
16	Efek Pemberian L-Arginin Terhadap Gambaran Histologi Jumlah Spermatisit Primer pada Mencit (Mus musculus) Setelah Terpapar Suhu Panas	2019
17	Anti Prolactine Overcomes Heat Stress on Laying Hen.	2008
18	Unnatural Forced Moulting in The Laying Hen as Cause of Zoonosis from Salmonella Enteritidis	2009
19	Case Study: Dystocia on Beef Cattle in Kunir Regency of Lumajang District, East Java, Indonesia in 2015 and 2016	2017
20	Teratogenic Effect of Congenital Toxoplasmosis in Chicken Embryo	2017

Adapun penelitian tersebut layak dilakukan, meskipun belum ada ***Ethical Clearence*** karena menggunakan hewan coba yang minimal dan menghasilkan output yang sangat baik.

Demikian surat keterangan ini kami buat untuk dapat dipergunakan sebagai persyaratan pengusulan Jabatan Fungsional **Guru Besar**

Surabaya, 3 April 2023

Wakil Dekan III,

Prof. Dr. Mustofa Helmi Effendi, drh., DTAPH

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Dear Erma Safitri,
Universitas Airlangga

Once again, I would like to congratulate you on your successful paper submission. This letter is an official confirmation that your paper

“Teratogenic Effect of Congenital Toxoplasmosis in Chicken Embryo”

is **ACCEPTED** to be presented at The **VMIC – Veterinary Medicine International Conference 2017** in Biomedical Science Oral Session 5 on Thursday 13th of July 2017 and is submitted to be published in our proceeding in **KnowledgeE** publisher.

Be sure to follow us on our social media platforms, and keep an eye on our website, to make sure you remain up to date with our upcoming conference! I would like to wish you luck with the registration procedures, as well as all the preparations for the upcoming conference to be held from the 12th to the 13th of July, 2017.

Do not hesitate to contact us with any questions or concerns. You can do this on VMIC website <http://conference.unair.ac.id/index.php/vmic/vmic1>, via mail vmic@fkh.unair.ac.id or by phone/Whatsapp Ms.Martia Rani Tacharina +62 87852341377 .

Kind Regards,

Chairman,

Prof. Dr. Fedik A. Rantam, DVM






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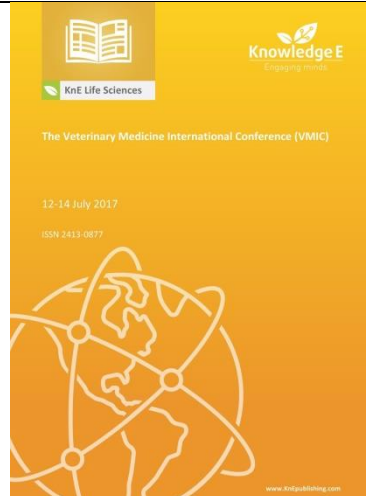
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Conference Paper

Teratogenic Effect of Congenital Toxoplasmosis in Chicken Embryo

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Abstract

This research is designed to observe the teratogenic effect of *Toxoplasma gondii* infection in chick embryos, based on the number of somites, embryo length and the development of embryonic brain vesicles. Methods in the research: Chicken eggs were infected with 1×10^3 tachyzoites of *T. gondii*. The eggs were incubated in eggs hatching box. Observation of somite performed on embryonated eggs 24 hours after incubation and the embryonic development of vesicles performed 72 hours after incubation then the length of each embryo were measured. Results: Revealed that there was a significant difference in the number of somites ($p < 0.1$), *T. gondii* infection reduced the number of somites. While in the number of brain vesicles in 3 - days old chicken embryos, although there was no significant difference, the size declining emerged. The length of the embryos both at 24 or 72 hours old showed that *T. gondii* infection reduced the length ($p < 0.1$). Conclusions: *T. gondii* infection influences the development of chicken embryos in the declining of length and the decreasing of somite embryo number.

Keywords: IGF-I crossbreed mare serum pregnant; Follicle; Mus musculus.

1. Introduction

Toxoplasmosis is a zoonotic disease that caused by the parasitic protozoan *Toxoplasma gondii* [1-4]. Humans, livestock and poultry were infected by sporulating oocysts that pollute the environment [5-6]. A human can also be infected by eating undercooked meat [7-8]. In pregnant mammals, both livestock and human, *T. gondii* infection can be transmitted transplacentally [9] and may risk the fetus to get fetal absorption, abortion, stillbirth, infant death and congenital abnormalities born weak, depending on the time of gestation getting an infection [10-12]. *T. gondii* infection, in livestock, is also given rise to problems such as fetal pathology and abortion [12]. *T. gondii* infection is a major cause of abortion goats and sheep in several countries including Australia

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and the United States. The frequency of occurrence of abortion and fetal death in sheep infected with *T. gondii* is quite high [13-14]. Studies on the incidence of toxoplasmosis in chickens and chicken eggs have been done by several researchers. The study includes serological and parasitological study and the results of these studies indicate the incidence of toxoplasmosis in chickens and eggs is quite high [15-17]

The risk of infection due to chicken embryos in eggs rarely have been reported. The aim of this research is to reveal the teratogenic effects of *T. gondii* infection in chicken embryos, based on the number of somites, the development of the embryonic brain vesicles and length of the embryo. It is expected that this study can be used as a research model of *T. gondii* infection teratogenic effects in mammals, including human embryos

2. Materials and methods

Forty chicken eggs were divided into two groups. The first groups as a control and the other infected with *T. gondii*. Isolates of *T. gondii* was RH strain. Infection procedures appropriated with [18-19]. Infection dose was 1×10^3 tachyzoites. tachyzoites injected into chorioallantois and then closed with paraffin and masking tape. After infection, all of the eggs were incubated in hatching box.

Teratogenic effects based on embryonic development, include: the number of somites in the embryo, the length of embryos and brain vesicle formation Isolation Embryos was done by using whole- mounts [20]. Ten embryonated chicken eggs of each group were observed at 24 hours and then the other at 72 hours after incubation. Embryonated chicken eggs were opened by eggshell peeling.

The egg was dipped in 0.9% saline buffer and simultaneity poured the contents of the egg. The red part in hollow eggs was containing an embryo. Embryonic membranes were cut using a glass slide and embryo was raised and put on the glass. Embryos were observed under a microscope. The embryonic length was measured from the anterior end to the posterior end of the embryo ages 24 and 72 hours after infection, a number of somites were calculated on embryo 24 hours old and the development of embryonic vesicles was done embryonated chicken eggs age of 72 hours.

The number and length of somite chicken embryos were analyzed by t test, while for the development of embryonic vesicles descriptively presented.

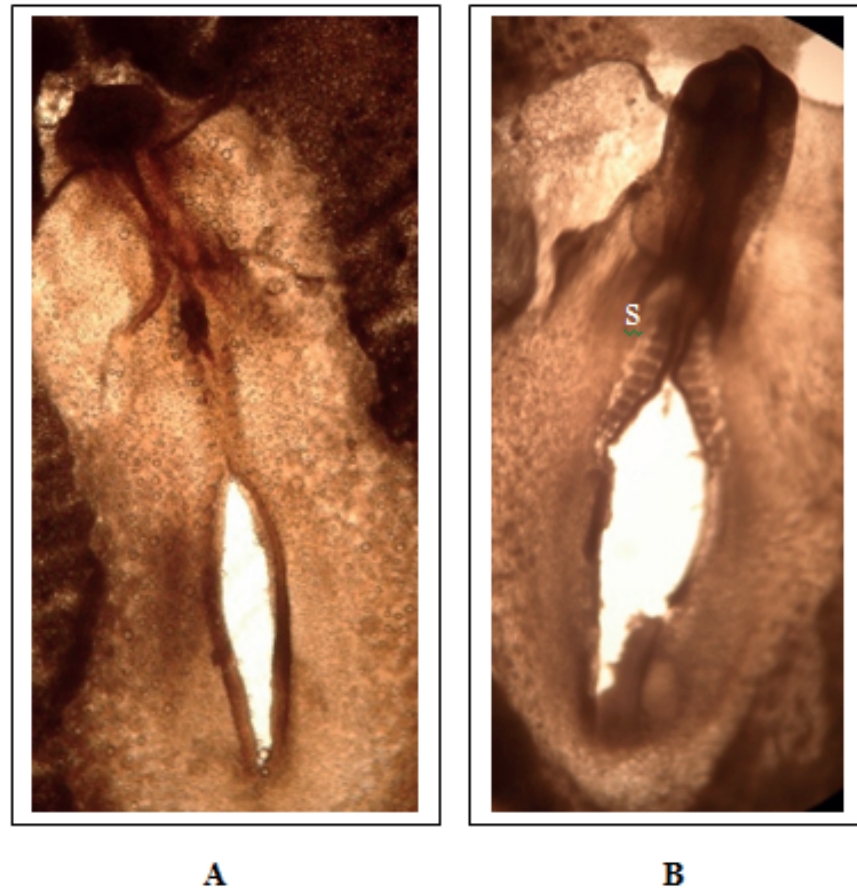


Figure 1: Chicken embryo after 24 hours incubation. **A.** Control . **B.** infected with *T. gondii* S: somite.

3. Results

From the statistical analysis, the number of somites was a significant difference ($p < 0.1$) between chicken embryos infected compared with uninfected. In infected embryos, the number of somites decreased from 12.8 (uninfected) become 9.4 (infected). (Table 1 and Figure 1).

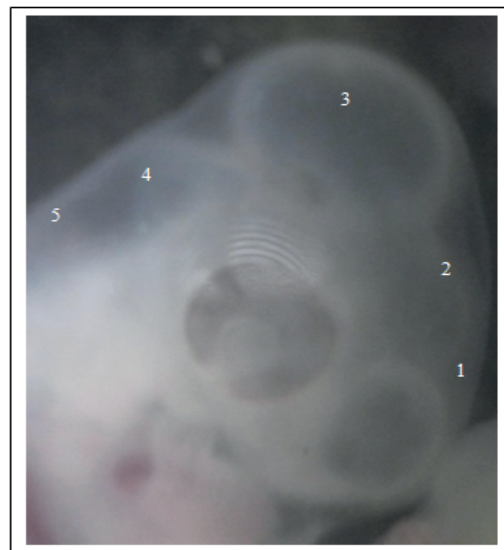
The results of the measurement of the length of the embryo on 24 hours and 72 hours showed that the infection *T. gondii* decrease the length of the embryo. In control groups (uninfected egg) was 0.56 cm and 1.80 cm but in infected eggs were 0.42 cm and 1.12 cm (Tabel 1).

In 72 hours old chicken embryos, both infected and uninfected, all vesicles in the brain are already complete, but brain size in infected group was significantly decreased compared with the control group (Figure 2).

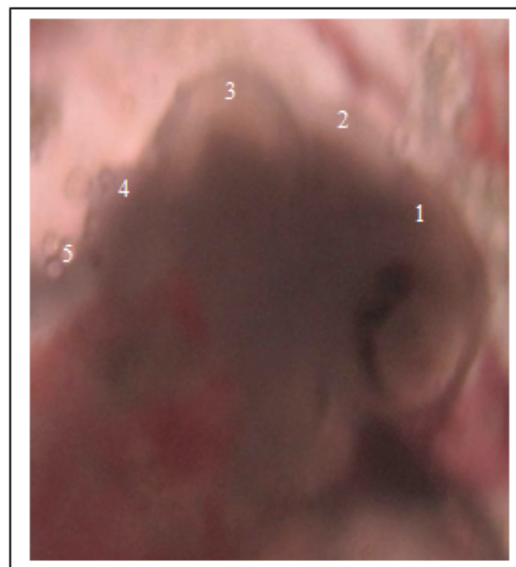
A somite is part of the embryo that will develop into skeletal muscles and skeleton, in the development of somite chick embryo begins to form in 21 hours after incubation

TABLE 1: Mean and Standard Deviation of number of somite and length chicken embryos 24 and 72 hours after incubation.

		number of somite (24 hours after incubation)	length embryo (cm)	
			24 hours after incubation	72 hours after incubation
Treatment	Control	12,80±0,87	0,56±0,05	1,80±0,20
	Infected	9,40±0,55	0,42±0,04	1,12±0,08



A



B

Figure 2: Barin of Chicken embryo after 76 hours incubation. **A.** Control (12,5x) . **B.** infected with *T. gondii* (40x) 1. Telencephalon, 2. Diencephalon, 3. Mesencephalon, 4. Myelencephalon, 5. Metencephalon.

[20]. In this study, observation of the number of somites was done in 24 hours after incubation. In infected group, the number of somites was fewer than an uninfected egg

(Table 1), it indicates that *T. gondii* infection has affected the development of chicken embryos since the first day (24 hours) after infection. This indication is the explanation of the declining of embryos length, both of which lasted for one day or three-day experience also decrease (Table 1).

Moreover, since skeletal muscle is a potential somite and skeletal, the somite number decreases when the length of the frame also decreases, consequently decreasing the length of the embryo as well. The decrease in the number and length of somite embryo is probably also the explanation of the results of previous research, which says that *T. gondii* infection causes a decrease in fetal mice [21].

The decreasing of the number of somite and length chick embryos may caused by Toxopain-1. Toxopain-1 is a member of a family of cysteine proteinase that plays a role in the pathogenesis of *T.gondii* infection [18]. This protein is a product rhoptry of *T. gondii* and it is released during *T. gondii* penetrate the host cell membrane to survive and multiply. Toxopain-1 plays a role in the pathogenesis of congenital toxoplasmosis, the research found that Toxopain-1 inhibited the development of chicken embryo manifested by weight of embryos and organs of the embryo and the embryo occurs more severe organ damage [18].

In the development of the chicken embryonic brain, differentiation has been perfect at 72 hours after incubation marked five vesicle formation which are: Telencephalon, Diencephalon, mesencephalon, Mielenchephalon, and Metecephalon. During the process of embryonic brain development, it is very susceptible to the presence of the teratogen agents [20].

Based on the number of brain vesicles of 3-day old chicken embryos, there was not the significant difference between groups of infected and uninfected eggs. The number of brain vesicles from both groups is complete, there are 5 vesicles emerged. It is shown that *T. gondii* infection does not affect against the differentiation of the brain. But judging from its structure, *T. gondii* infection may cause the decreasing of the brain size (Figure 2).

From the results, it can be concluded that there was no effect of *T. gondii* infection in the formation of brain vesicles and it can not be used as an occurrence marker of teratogenic effects of infectious agents. Teratogenic effects on embryonic development has to be thoroughly observed in it's all parameters which are somite number, the length of the embryo, embryo weight and death embryo.

Toxoplasma gondii infection affects the development of the chick embryo by the decrease in the number and length of somite embryos and Toxoplasmosis infection had no effect on chicken embryonic brain vesicle formation.

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