

## **EFFECT OF SOMATIC ANISAKIS SIMPLEX EXTRACT TO DEVELOPMENT CHICK EMBRYOS**

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### **ABSTRACT**

Helminthes have embryotoxic and teratogenic effects to host's cells and tissues. Investigations were carried out mainly on embryo and fetus in mammals. Blaszkowska, J. (1998); Kadlubowski R. (2000) conducted studies of in chick embryos. Dose-dependent effect is also studied and found that small doses cause embryotoxic effect, while higher doses - teratogenic. *Anisakis simplex* antigens have also embryotoxic effect has been found. The aim of our research was to examine the effect of *A. simplex* somatic antigen to the development of chick embryos in its experimental introduction in various ways and at different stages of embryogenesis. For the study chicken embryos were used at different stages of embryogenesis: 7.5, 10.5 and 12.5 days. Methods for introducing antigen chicken embryos: in the allantois cavity, on-chorion allantois membrane and yolk sac at 0.2 ml. The autopsy was performed on embryos 2 and 8 days. The eggs and embryos were weighed and evaluated the embryonic development. As a result of the first experience the greatest change observed by mass, and when administered in the development of antigen in both yolk sac on day 2 ( $1.68 \pm 0.06$ , unlike control  $2.25 \pm 0.20$ ), and at 8 days ( $9.97 \pm 6.21$  in the experiment versus  $14.89 \pm 0.78$  in the control), respectively. In the second experiment eggs were used at 1 and 5 day incubation embryos. The antigen was administered in both cases also in the yolk sac at a dose of 0.2 ml. In the second experiment there was a delay in the development of both cases at autopsy after 2 days in non-incubated eggs missing the development of, unlike the control, the development of which 48 hours corresponded; including 5 daily observed decrease in weight  $0.88 \pm 0.22$ , against  $1.05 \pm 0.05$  and 8 days in a similar way. As a result of experiments on the effect of the *Anisakis simplex* somatic antigen on the development of chick embryos embryotoxic action installed. Thus antigen has the greatest effect on development of early embryos and putting it into the yolk sac.

**Keywords:** *Anisakis simplex* antigen, chicken embryos, embryotoxic, teratogenic.

### **INTRODUCTION**

Helminthes and their metabolites have embryotoxic and teratogenic effects to host's cells and tissues. *Anisakis simplex* antigens have also embryotoxic effect (Sivkova and Berezhko, 2011) Scientists have conducted research mainly on

embryos and fetuses in mammals (Bekish et al., 2010; Blaszkowska, 1998a; 2000; 2008; Kadlubowski). Researchers conducting similar experiments on chick embryos are very few (Blaszkowska, 1998b, Kadlubowski and Blaszkowska, 2000). The researchers studied the dose-dependent effect and found that small doses cause embryotoxic effect, while higher doses - teratogenic. Embryotoxic effect is accompanied by a decrease in weight and / or death of the embryo.

Chicken embryos for the study of vertebrate embryogenesis are very comfortable model, because there is no harm to the mother's body. For research may be applied various methods: embryonic, histological and cytological and other (Trunova, 2008). The aim of the study was to investigate the influence of *A. simplex* somatic antigen to the development of chick embryos in the experimental introduction of a variety of ways and at different stages of embryogenesis.

### MATERIALS AND METHODS

For the study chicken embryos were used at different stages of embryogenesis: 7.5, 10.5 and 12.5-days. Egg was one of the same parties, the breed Ross-308. Automatic Incubator Microprocessor-controlled RCom 50 Pro (South Korea) was used for the incubation of eggs. We used the following incubation conditions: temperature (37.4-37.8° C), humidity (60-70%), ventilation and turning the eggs every hour, was created for the development of chicken embryos. Ovoscope was used to control the experiment and assess the viability of the embryo.

The investigated *A. simplex* extract was injected to chicken embryos according to the standard method (Bliieva, 2010). Before the introduction of the antigen eggs appeared through, checking the availability of contribution of blood vessels of the embryo and have noted pencil borders pneumatic bag, the location of the embryo and place of antigen injection. Injection of the antigen was performed in aseptic conditions using of a sterile instrument. Disinfection of eggs is performed by treatment with 70% ethyl alcohol solution and by flame. The *A. simplex* extract was injected with a sterile insulin syringe and a sterile needle. Place of injection were sealed sterile tissue adhesive plaster, and then embedded in paraffin. For the study chicken embryos were used at different stages of embryogenesis: 7.5, 10.5 and 12.5 days. Methods for antigen introducing to chicken embryos: in the allantois cavity, on-chorion allantois membrane and yolk sac at 0.2 ml. For each method of administration of the extract and control were taken at three chick embryo.

After injection of the *A. simplex* extract embryos were placed to the incubator. The autopsy was performed on embryos 2 and 8 days, pre for 4-6 hours at 4°C embryos were placed into the cold. The eggs and embryos were weighed and evaluated the embryonic development. Egg's shell is treated with alcohol and removed gently, examined on-chorion allantois membrane, amniotic fluid and removed embryo. Chicken embryo was evaluated age and its degree development during incubation, by comparing the standard values (Dyadichkina et al., 2010). Control remained intact biocontrol conducted during the experiment to monitor the development of chicken embryos. For the second experiment we used 1-day and 5-day chick embryos. In both cases the antigen injected at a dose of 0.2 ml in the yolk sac.

## RESULTS AND DISCUSSION

In the intact group changes are not were observed, the weight, the age of the chicken embryo and the degree of their development corresponded to established standards. In the experimental group were observed changes in weight, results are presented in Table 1.

Table 1. Development of 7-12-day-old chick embryos after influence of *A. simplex* somatic antigen extract

Age of chick embryos, day	2 days Incubation			
	Control		Experience	
	egg weight, g	embryo weight, g	egg weight, g	embryo weight, g
7,5	56,35±0,30	2,25±0,20	54,07±0,49*	1,68±0,06*
10,5	53,40±1,05	5,48±0,03	54,87±0,31	6,13±0,21*
12,5	56,08±4,23	14,00±0,85	55,87±0,79	13,83±0,82
8 days Incubation				
7,5	59,83±1,78	14,78±0,78	56,27±0,91	9,97±6,21
10,5	54,13±1,38	26,6±1,25	55,83±0,26	26,33±0,39
12,5	54,30±0,90	46,60±0,75	52,80±1,50	44,58±1,38

\* 0,05

We have found that in the control after two days of incubation in 7.5 day embryos which antigen was injected into the yolk sac of development corresponds to 9 days. They were formed: a curved beak, egg tooth forever elliptical shape, and the beginnings of feathers. Chicken embryos lagged behind in development, were visually less in experience than in the controls.

Two days later the embryos dissected and evaluated their age. Embryos after antigen injection into the allantois cavity corresponded to 11 days incubation in the control. In the experiment the age and development of chicken embryos match to 12 days of incubation.

At chick embryos observed slight decrease in weight in contrast to the control. In embryos were clearly visible claws on his fingers,  $\frac{3}{4}$  cornea was covered with the lower eyelid, the opening between the eyelids in the form of a narrow slit.

In the control group it was found 12.5-day embryos were which antigen injected in yolk sac, the development corresponded to 14-15 days after two days incubation. We were observed yolk sac has been reduced, the eyelids were closed. We found that in the experience, not all at in embryos were closed eyelids; their age corresponds to 13 days.

We have discovered that 7.5-day-old embryos in the control after eight days of their development corresponded to 15 days. The yolk sac has been reduced, the amount of allantois fluid was increased, the protein tunica was very small and the eye lids were closed. However, it was noted that in experiment in one embryo the development was absent, namely stopped for 6-7 days. It was observed that the

embryo's beak has been pushed forward, egg tooth is missing, the second finger of the wing was longer than the others, the toes are webbed, and the mass was less than one gram to contrast to other the rest embryos, mass was which 14.0 grams (Fig.1 ).



Figure 1. 7.5 day embryos, after *A.simplex* antigen introduction of in the yolk sac, the incubation of 8 days

With the antigen injection into the allantois cavity of 10.5 daily, developing chick embryos controls correspond to 18 days. In the control group all the embryos head was under the right wing, and was sent to the air chamber. In the experiment we found that one embryo of the head was in the opposite direction that is downward, in other embryos head is also directed towards the air chamber, but are not under the right wing, a little lagged behind at development.

In control 12,5- days embryos conform by age 20 days. We found when compared control with experiment in which at two embryos was not hole at on-chorion allantois membrane, at one embryo was not retracted yolk sac, and was also allantois fluid, which in this should not be aged. In the second experiment, the following results were obtained Table 2.

Table 2. Development of 1-5-day-old chick embryos after *A. simplex* somatic antigen extracts influence

Age of chick embryos, day	2 days Incubation			
	Control		Experience	
	egg weight, g	embryo weight, g	egg weight, g	embryo weight, g
1	64,40±1,70	-	62,73±1,21	-
5	60,98±2,13	1,05±0,05	61,80±2,20	0,88±0,22
8days Incubation				
1	66,88±0,17	1,43±0,08	65,87±2,86	0,40±0,50
5	60,63±0,23	11,70±0,40	64,17±1,08*	9,10±3,27

\* 0,05

In the control group was found after 48 hours where in the antigen injected into the yolk sac 1-day chicken embryos their development correspond to 48 hours of incubation, blastodisk was formed, were visible blood vessels, which is the norm. In the experiment it was found the lag in the development. The age of two embryos

corresponded to 24 hours (Fig. 2), and observed in an egg yolk uneven coloration and was a gas bubble.

After 2 days, we evaluated the age of 5-day-old embryos in the control group corresponded seven days of incubation; and in the experimental group at one embryo weight was 2-fold less than the other (Figure 3).



Figure 2. Experiment 1-day chicken embryos injection *A.simplex* antigen 2 days incubation, development corresponds to 24 hours



Figure 3. Experiment 5-day chicken embryos injection antigen *A.simplex* 2 days incubation, the arrow shows the embryo lagging by weight

The autopsy revealed that the age of 1-day embryos after 8 days of incubation in control age was 7-8 days. We did not observe any changes in the development of. In the experimental group, development stopped at two chicken embryos 72 hours and 48 hours; one embryo was observed lag by weight (Fig. 4).

After 8 days, we did not detect changes in development among the 5-day embryos in control; they were on the description 13 days. In the experience of the development has stopped with one embryo for 7-8 days. He had the characteristic shape of the head, beak, feathers beginnings, he was prone to maceration, and his cloths were dirty pink and flabby, due to hemolysis (Fig. 5).

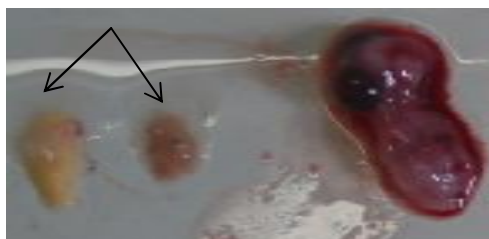


Figure 4. Experience 1-day chicken embryos under the *A.simplex* antigen action, incubation of 8 days, and the arrows indicate the embryos whose development stopped at 72 and 48 hours of incubation



Figure 5. Experience 5-day chicken embryos under the *A.simplex* antigen action, incubation of 8 days

## CONCLUSION

As a result of these experiments is set:

1. *Anisakis simplex* somatic extract has embryotoxic effect on embryo chicken eggs;

2. Chicken embryos were more sensitive to *A. simplex* somatic extract 1-5 days of embryonic development.

3. *A. simplex* somatic extract has a greater impact on chicken embryos when injected into the yolk sac, than when it is introduced into the allantois cavity, on chorion allantois membrane.

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