

## **Observation on the *in-vivo* administration of oxytetracycline solution in partridge egg**

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### **ABSTRACT**

*Microorganisms are often the cause of many infectious diseases in human, animals and birds. In recent years, antibiotics are used in veterinary medicine to treat different types of infections. They injected into hatching eggs to eliminate pathogens and prevention of egg transmission of disease, but the adverse effects of drugs have always been a major concern. There is scanty information available about the safety and pathological alterations of tetracycline drugs in the embryonated eggs of the game birds. The objective of this study was to investigate using of oxytetracycline injectable solution for in ovo administration in partridge embryo. Fertile partridge eggs were divided into three equal treatment groups as follows: uninjected group, phosphate buffered saline-injected group and oxytetracycline-injected group whose individuals were injected with oxytetracycline injectable solution at a dosage of 10 mg per Kg egg-weight. Embryos were re-incubated post-treatment and allowed to develop until day 21 after which; they were examined for macroscopic and microscopic lesions. Results showed that embryos were normal in all treatment groups. Microscopically, no lesions were also diagnosed in tissues. Based on macroscopic and microscopic findings, it is concluded that oxytetracycline at above-mentioned concentration is not toxic for the partridge embryo. So, oxytetracycline egg-injection can be used to eliminate pathogens and prevention of egg transmission of the disease without any adverse effect.*

**Keywords:** Embryo, Histopathology, Oxytetracycline, Partridge

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### **INTRODUCTION**

Bacterial diseases resulted in significant economic losses in the game bird industry. Methods include development in the management protocols and using different types of antibiotics were applied to alleviate economic losses in this industry [36, 42].

Antibiotics have been used across the globe for many years. Today, they are used on a large scale and are applied for different purposes [8, 10, 11, 38, 39]. In veterinary medicine, they are used to prevent disease, cure animals and birds, or as a feed additive to promote growth.

Oxytetracycline is a broad-spectrum synthetic antibacterial agent that is effective against most gram-positive and gram-negative bacteria isolated from domestic poultry and animals. It belongs to the tetracycline antibiotics group, and is indicated in treating serious infections due to susceptible strains of pathogens. It is often dispensed as an aid for preventive and therapeutic treatment of gastrointestinal and respiratory tract infections, caused by oxytetracycline sensitive microorganisms such as *Chlamydia*, *Pasteurella*, *Haemophilus*, *Enterobacter*, *Escherichia*

*coli*, *Salmonella*, and *Diplococcus*. Oxytetracycline inhibits cell growth by inhibiting translation. It binds to the 30S ribosomal subunit and prevents the amino-acyl tRNA from binding to the A site of the ribosome. Oxytetracycline is also lipophilic and can easily pass through the cell membrane or diffuses through porin channels in the bacterial membrane. [24].

Birds treated with oxytetracycline show better body weight gains and better feed utilization during growth period. Additionally, prophylactic application of oxytetracycline in the game bird farms, decrease the mortality rate. In hatcheries, the hygienic process in association with injecting antibiotics into the egg, result in eliminating infection and preventing egg transmission of pathogens. Alternatively, eggs may be dipped in the antibiotic solution for controlling the disease transmission before hatching.

Adverse effects of drugs have always been a major concern. There is little research in the literature describing the effect of antibiotics on the developing bird embryos, and further studies still need to be undertaken to determine the safety, toxicity and teratogenic potential of antibiotics. On the other hand, the application of antibacterial drugs for in ovo administration in the game bird's egg still needs to be justified. In this regard, in the present study, we investigated using of oxytetracycline solution for in ovo administration in embryonated partridge eggs. We believe that results in this study will contribute to our better understanding of safety and pathological effects of tetracycline drugs on the game bird embryos.

## MATERIALS AND METHODS

### Hatching eggs

Fertile partridge eggs (Chukar partridge) with the average egg-weight of  $21 \pm 0.6$ g and with the same age were purchased from a local breeder farm. In this farm, birds were kept and grown up under the standard condition of breeding.

### Drugs

Oxytetracycline 5% injectable solution was obtained from the Razak Pharmaceutical Company, Iran. Each milliliter of drug contains 50 mg oxytetracycline. It was diluted in phosphate buffered saline solution. A volume of 0.5 mL of phosphate buffered saline solution with 10 mg oxytetracycline was inoculated per Kg egg-weight.

### Experimental protocol

Eggs were incubated at 37.5°C and 55% relative humidity. The eggs were randomly assigned to three equal treatment groups, 10 eggs each, as follows: group 1: uninjected group; embryonated eggs do not receive any treatment at all. Group 2: phosphate buffered saline injected group, embryonated eggs injected with sterile phosphate buffered saline of 0.5 ml/egg into the yolk sac. On day 4 of incubation, the eggs of group 3 treated with oxytetracycline injectable solution at a dosage of 10 mg oxytetracycline per Kg egg-weight. Embryos received treatment by direct injection into the yolk sac according to the standard techniques [13]. Embryos were re-incubated post-treatment and allowed to develop. The viability of the embryos was checked throughout the incubation period by candling. All embryos were necropsied on day 21 of incubation and examined for macroscopic and microscopic lesions. The treatment protocols and procedures in this study were conducted according to local ethical guidelines, and were approved by the Animal Ethics Committee of the Research Council of Shahid Bahonar University, Iran.

### Pathological examination

At the end of the experiment, on day 21, embryos were humanely killed by placing on ice and then the eggs were opened at the wider end [15]. After washing in normal saline solution, embryos were observed under stereomicroscope to study any gross abnormalities on the external body surface. The membranes and yolk sac were also inspected. Then, the tissues of embryos were dissected out and fixed in 10% neutral buffered formalin. Following routine preparation of tissues, serial sections of paraffin embedded tissues of 5  $\mu$ m thicknesses were cut using a microtome (Slee-Germany) and stained with hemotoxylin and eosin and studied under light microscope.

### Statistical analysis

Statistical analysis was performed using SPSS version 20. The Chi-Square test was used to determine the significant differences in lesion occurrence between experimental groups. A P-value of  $<0.05$  was considered as statistically significant.

## RESULTS

### Macroscopic results

The tissues of the embryos were normal in groups 1 and 2 (figure 1). In oxytetracycline-injected groups, group 3, there was not any gross abnormality in the tissues and external body surfaces (figure 2). The obtained tissue samples of these embryos were sent to the pathology laboratory.



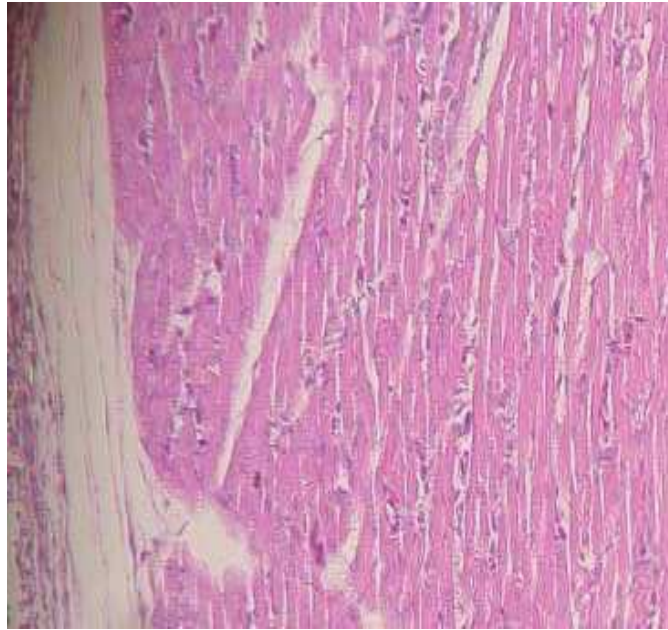
**Fig. 1.** The partridge embryo treated with phosphate buffered saline solution into the yolk sac. The embryo is normal with no gross lesions



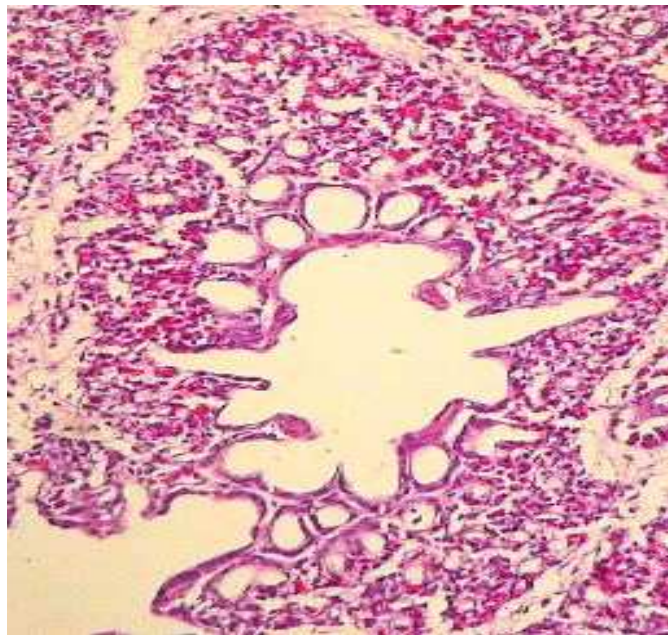
**Fig. 2.** The partridge embryo treated with oxytetracycline injectable solution into the yolk sac. The embryo is normal with no gross lesions

### Microscopic findings

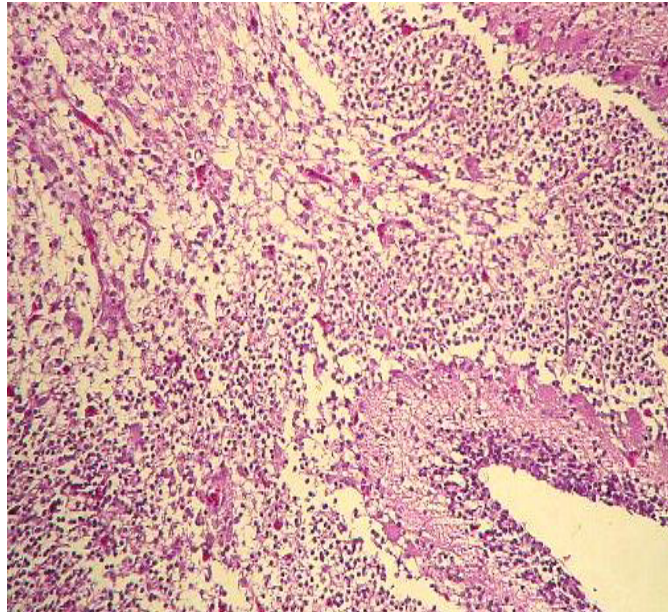
Histopathological evaluation has been revealed that all organs were normal in groups 1 and 2. In embryos of group 3, which received the oxytetracycline injectable solution, all microscopic structures were also normal (figures 3-8).



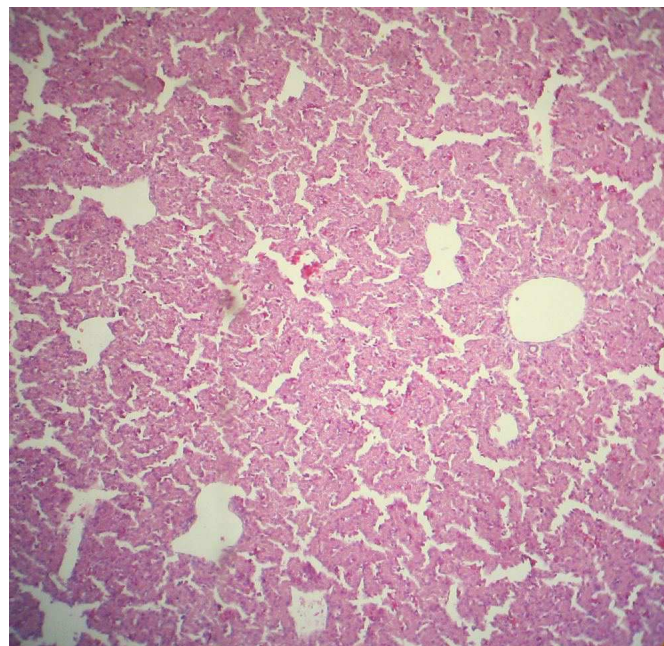
**Fig. 3.** Photomicrograph of the partridge embryo treated with oxytetracycline injectable solution into the yolk sac. A normal structure of the heart is seen.  $\times 100$  H&E



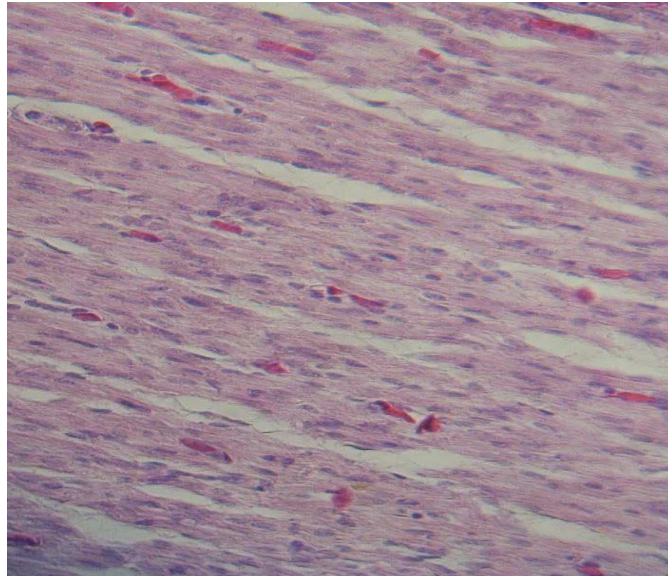
**Fig. 4.** Photomicrograph of the partridge embryo treated with oxytetracycline injectable solution into the yolk sac. A normal structure of the lung is seen.  $\times 100$  H&E



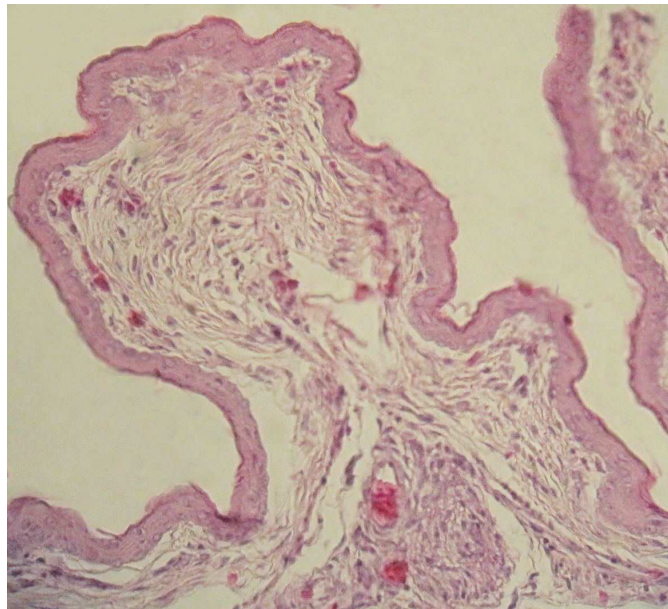
**Fig. 5.** Photomicrograph of the partridge embryo treated with oxytetracycline injectable solution into the yolk sac. The normal structure of the cerebellum is seen. ×400 H&E



**Fig. 6.** Photomicrograph of the partridge embryo treated with oxytetracycline injectable solution into the yolk sac. The normal structure of the liver is seen. ×100 H&E



**Fig. 7.** Photomicrograph of the partridge embryo treated with oxytetracycline injectable solution into the yolk sac. The normal structure of the muscle is seen.  $\times 400$  H&E



**Fig. 8.** Photomicrograph of the partridge embryo treated with oxytetracycline injectable solution into the yolk sac. The normal structure of the skin is seen.  $\times 400$  H&E

## DISCUSSION

The game bird industry has experienced tremendous development and expansion during the past ten years. On the other hand, pathogenic agents are an important and significant hazard for poultry health and cause serious economic losses to this industry. For many years, researchers have been using different antibacterial compounds to restrict pathogens and enhance the performance of different poultry species, including young chicken [4, 6, 29, 32], quail [7, 23, 31], turkey [3, 5, 9], broiler [1, 19, 21, 25, 41], layers [14, 20, 28] and poultry breeder [16, 17, 30, 42].

Tetracyclines have an increased role as therapeutic agents against avian pathogens. They have bacteriostatic effect and a wide antibacterial spectrum. Most gram-positive and gram-negative organisms are susceptible [2, 37]. Oxytetracycline belongs to the tetracycline pharmacological group. It has been used successfully for several decades in many countries such as Canada, Spain, France, Austria, Polish, Denmark, Germany, Turkey, Africa, United States and China. In recent years, its use has increased rapidly in the Iranian poultry industry, but there is little information available about the effects of injecting oxytetracycline injectable solution into the game bird's egg. Besides, determining the side effects of drugs on the development of bird embryo is a useful method for studying the

biological properties of drugs. In the present study, we investigated the using and toxicity of oxytetracycline solution for in ovo administration in partridge egg. Lesions and organ injuries following administration were also inspected. Up to now, antibiotic-egg-treatment has been examined and described in different situations [12, 18, 26, 33-35, 40]. The results of these studies show that injecting antibiotics into hatching eggs can eliminate pathogens and prevent vertical transmission of disease. Some antibiotics such as tylosin and gentamicin were effective in reducing egg-transmission of infection [26, 27]. Tylosin was used because of its efficiency against mycoplasmas and gentamicin was used because of its broad-spectrum activity against bacteria and its low toxicity to host cells. Dosage and the rout of injection can have an influence on the outcome. For example, tylosin can be toxic for eggs when used in high doses [27]. On the other hand, some injection sites that are present in fertile eggs at day 4 of incubation are the air cell and yolk sac. Injection antibiotics into the air cell of the egg is discontinued and is not suitable for breeding purposes because drastic mortality of embryos occur when eggs treat by this procedure [22, 27].

Our results obviously showed no gross abnormality in the tissues and external body surfaces of the partridge embryos exposed to oxytetracycline solution by yolk sac rout. Histopathological examination has also been revealed that all organs were normal in embryos. Therefore, these results suggest that the best oxytetracycline injection sites in ovo may be the yolk sac. Nevertheless, further efforts are needed to evaluate in ovo administration of various tetracycline drugs for prevention and eliminate pathogenic microorganisms.

In conclusion, based on macroscopic and microscopic findings, it is concluded that oxytetracycline solution can be used for the success of the eradication scheme with low toxicity to partridge embryo. In addition, the yolk sac is an appropriate site for injecting antibacterial drugs.

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