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Original Article

The Effect of Doxycycline on the Viability of the Quail Embryo During Incubation Period

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ABSTRACT

Objective: In recent years, antibiotics have been used widely in the glob to prevent and treat bacterial diseases. They injected into 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 little information available about the safety of tetracycline drugs in the embryonated eggs of the birds. The objective of this study was to evaluation of the effect of doxycycline on the viability of the quail embryo during the first trimester of the incubation period. **Methods:** Fertile quail eggs were divided into two equal treatment groups. The uninjected group and doxycycline-injected group whose individuals were injected with doxycycline injectable solution at a dosage of 10 mg per Kg egg-weight. Embryos monitored daily during incubation until day 6 after which; they were examined for viability. **Results:** Results showed that 72% of embryos were alive in the doxycycline-injected group. Based on findings, it is concluded that doxycycline at above-mentioned concentration has no severe lethal effect on the quail embryo. So, doxycycline egg-injection can be used to eliminate pathogens and prevention of egg transmission of the disease.

1.INTRODUCTION

The quail farming industry began in the glob around the early 1970s. Raising quails for their eggs and meat is becoming a popular and lucrative business in many countries including Iran. Bacterial diseases are the most common and destructive disease of the quail (Swayne et al. 2013). So, different types of antibiotics were used to prevent and treatment of bacterial diseases in this industry (Swayne, Glisson et al. 2013; Tavakkoli et al. 2104). Doxycycline is a long-acting tetracycline antibacterial agent that is effective against most gram-positive and gram-negative bacteria. It belongs to the tetracycline antibiotics group, and is used in prophylaxis against most strains of pathogens. It is also effective against, skin and soft tissue infections, ophthalmic,

gastrointestinal and respiratory tract infections, caused by doxycycline sensitive microorganisms such as, *Listeria*, *Staphylococcus*, *Bacillus*, *Streptococcus*, *Chlamydia*, *Pasteurella*, *Haemophilus*, *Enterobacter*, *Escherichia coli*, and *Salmonella*. At subantimicrobial dosages, doxycycline is an inhibitor of matrix metalloproteases, and has been used in various experimental systems for this purpose. Doxycycline is lipophilic and can pass through the lipid bilayer of bacteria. Doxycycline reversibly binds to the 30 S ribosomal subunits and possibly the 50S ribosomal subunit(s), blocking the binding of aminoacyl tRNA to the mRNA and inhibiting bacterial protein synthesis (Sweetman et al. 2009; Ahrens et al. 2013). In veterinary medicine, tetracycline is used to prevent disease, cure animals and birds, or as a feed additive to promote growth (Ahrens and Martin 2013; Swayne, Glisson et al.

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2013). 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 tetracycline on the viability of bird embryos, and further studies still need to be undertaken to determine this aspect. In this regard, in the present study, we evaluated the effect of doxycycline on the viability of the quail embryo during the first trimester of the incubation period. We believe that results in this study will contribute to our better understanding of safety of tetracycline drugs for in ovo administration in the bird's egg.

2. MATERIALS AND METHODS

2.1. Drug and eggs

Doxycycline 10% solution was obtained from the Rooyan Darou Pharmaceutical Company, Iran. Each milliliter of drug contains 100 mg doxycycline. It was diluted in phosphate buffered saline solution. A volume of 0.3 mL of phosphate buffered saline solution with 10 mg doxycycline was inoculated per Kg egg-weight. Fertile quail eggs (Japanese quail) with the average egg-weight of 10 ± 0.5 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.

2.2. Experimental protocol

Eggs were incubated at 37.5°C and 55% relative humidity. The eggs were randomly assigned to two equal treatment groups, 25 eggs each, as follows. Group 1: uninjected group; embryonated eggs do not receive any treatment at all. On day 2 of incubation, the eggs of group 2 treated with doxycycline injectable solution at a dosage of 10 mg doxycycline per Kg egg-weight. Embryos received treatment by direct injection into the yolk sac according to the standard techniques (Hamburger 1942). Embryos were re-incubated post-treatment and allowed to develop. The viability of the embryos was checked throughout the incubation period by candling. At the end of the experiment, on day 6, the eggs were opened at the wider end and monitored for the viability of the embryos. 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.

2.3. Statistical analysis

Statistical analysis was performed using SPSS version 20. The Chi-Square test was used to determine the significant differences in embryo-viability between experimental

groups. A P-value of <0.05 was considered as statistically significant.

3. Results

Candling of the hatching quail eggs during incubation period and examination of the eggs or embryos is a useful tool for hatchery managers. In the current study, the developing blood vessels were seen by candling of the eggs at the early stage of incubation (figure 1). In the next phase of incubation, the more advanced circulatory system was developed. Results of our experiments showed that the developing blood vessels were seen in 72% and 80% of quail eggs received no treatment and treated with doxycycline, respectively. At the end of the experiment, on day 6, the eggs were opened at the wider end and monitored for the viability of the embryos. As can be seen in the figure 2, there was centrally located embryo, the circulatory system and the blood vessels of the extra-embryonic membranes in the quail egg treated with doxycycline solution. Similar structures were also seen in the quail eggs received no treatment.

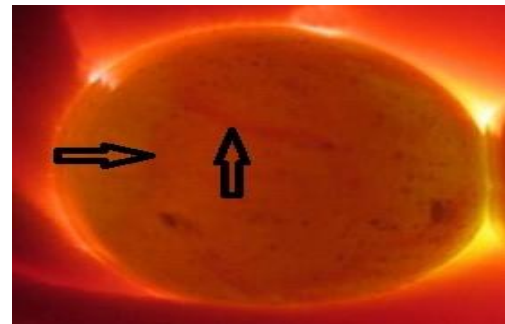


Fig. 1. The developmental age of a normal quail embryo. The developing blood vessels are seen (arrows).



Fig. 2. The quail egg treated with doxycycline into the yolk sac. A centrally located embryo, the circulatory system and the blood vessels of the extra-embryonic membranes are seen.

4. DISCUSSION

Despite reports of an expanding quail industry across the globe, pathogenic agents can decrease the hatchability rate during embryonic development. Embryonic death causes serious economic losses to the poultry industry (Swayne, Glisson et al. 2013). For many years, researchers have been using different antibacterial compounds to restrict pathogens and enhance the performance of different poultry species, including young chicken (Colomer-Lluch et al. 2011; Sapkota et al. 2011; Obeng et al. 2012; Banerjee et al. 2013), quail (McDougald et al. 2012; Crespo et al. 2013; Rigobelo et al. 2013), turkey (Altunsoy et al. 2011; Erdem et al. 2012; Buscaglia 2013), broiler (MacDonald et al. 2011; Agunos et al. 2012; Lee et al. 2012; Mosallanejad et al. 2014a; Tavakkoli et al. 2014b), layers (Hasan et al. 2011; Lee et al. 2013; Nemati 2013) and poultry breeder (Kabir 2010; Priyantha et al. 2012; Jones et al. 2013; Tavakkoli, Derakhshanfar et al. 2104).

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 (Sweetman, Pharm et al. 2009; Ahrens and Martin 2013). Doxycycline 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 doxycycline solution into the game bird's egg. In the present study, we have evaluated the effect of doxycycline on the viability of the quail embryo during the first trimester of the incubation period. Up to now, antibiotic-egg-treatment has been examined and described in different situations (Ghazikhanian et al. 1980; Sheeks et al. 1992; Kleven 2008; Singroha et al. 2012; Singroha et al. 2013; Mosallanejad et al. 2014b; Tavakkoli et al. 2014a). 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 (Nascimento et al. 2005; Mosallanejad, Tavakkoli et al. 2014b). 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 (McCapes et al. 1977; Nascimento, Pereira et al. 2005).

Our results obviously showed no severe lethal effect was seen in the quail eggs treated with doxycycline solution by the yolk sac route. Therefore, these findings suggest that the best doxycycline 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 findings, it is concluded that doxycycline can be used for the success of the prevention and treatment scheme with low adverse effect to the quail embryo.

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