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Efficiency of the injection of trimethoprim/sulfamethoxazole solution on game bird embryonated-egg during the late stage of development

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Abstract

Infectious agents cause disease in virtually any susceptible hosts. In the poultry production and medicine, pathogens were eliminated from a line of a breeder by injection of antibiotics into hatching eggs. There is little information available describing the safety of the folate antimetabolite/sulfonamide class of antibiotics on the game birds embryonated eggs. The objective of this study was to investigate the efficiency of the injection of trimethoprim/sulfamethoxazole solution on the partridge embryonated egg. Fertile partridge eggs were distributed into 3 groups and set in the incubator. On 18th day post incubation, one group was injected with the trimethoprim/sulfamethoxazole injectable solution dissolved in 0.3 ml phosphate buffered saline. Rest two groups were used as sham control (0.3 ml phosphate buffered saline solution) and un-injected control. Macroscopic evaluation on 21th day post incubation showed that embryos were normal in all treatment groups. Microscopically, no lesions were also diagnosed in the brain, heart, muscle, liver, kidney and lung of the embryos. Based on macroscopic and microscopic findings, it is concluded that trimethoprim/sulfamethoxazole at above-mentioned concentration is not toxic for the partridge embryo at the late stage of development. So**,** trimethoprim/sulfamethoxazole egg-injection can be used to eliminate pathogens and prevention of egg transmission of the disease without any adverse effect.

Keywords: Embryo, Histopathology, Trimethoprim/sulfamethoxazole, Partridge

Introduction

Pathogenic bacteria are single-celled microorganism can cause many serious diseases and economic cost throughout the world. They also resulted in significant economic losses in the game bird industry. Antibiotics are potent products that fight bacterial infection. They have always been considered across the globe. Today, they are used on a large scale and are applied for different purposes (Gharaibeh and Al-Rashdan 2011; Dubb 2012; Garner et al. 2013; Tavakkoli et al. 2014). Antibiotics have also been used to treat and prevent disease or promote feed efficiency in poultry and animal production. Trimethoprim/sulfamethoxazole belongs to the folate antimetabolite/sulfonamide class of antibiotics and is on the World Health Organization's list of essential medicines. It is effective in a variety of upper and lower respiratory tract infections, renal and urinary tract infections, gastrointestinal tract infections, skin

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and wound infections, septicaemias, and other infections caused by sensitive gram-positive and gram-negative bacterial agents such as *Acinetobacter, Enterobacter, Aeromonas, Proteus, Brucella, Vibrio, Staphylococcus, Streptococcus, Chlamydia, Campylobacter, Shigella and Mycobacterium*. Trimethoprim serves as a competitive inhibitor of dihydrofolate reductase (DHFR). The synergy effect is between trimethoprim and sulfamethoxazole drugs and they have a greater effect when given together. Sulfamethoxazole, a sulfonamide, induces its therapeutic effects by interfering with the synthesis of folate inside pathogens such as protozoa, fungi and bacteria. It does this by competing with p-aminobenzoic acid (PABA) in the biosynthesis of dihydrofolate. (McKellar et al. 2004).

In hatcheries, the hygienic process in association with injecting antibiotics into the egg, result in eliminating infection and preventing egg transmission of pathogens. 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 trimethoprim/sulfamethoxazole 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 folate antimetabolite/sulfonamide drugs on the game bird embryos.

Materials and Methods

Drug

Trimethoprim/sulfamethoxazole injectable solution was obtained from the Pantex Pharmaceutical Company, Netherlands. Each milliliter of drug contains 40 mg trimethoprim and 200 mg sulfamethoxazole. It was diluted in phosphate buffered saline solution. A volume of 0.3 mL of phosphate buffered saline solution with 20 mg trimethoprim and 100 mg sulfamethoxazole was inoculated per Kg egg-weight.

Injection protocol and pathological examination

Fertile partridge eggs (Chukar partridge) from the partridge breeders which are maintained in the standard condition and the adequate nutritional plan, with the average egg-weight of 20 ± 0.8, were collected from a local breeder farm. The eggs were randomly divided into 3 groups, 10 eggs each, and placed group wise in an incubator at 37.5ºC and 55% relative humidity. The first group was injected with trimethoprim/sulfamethoxazole injectable solution at a dosage of 20 mg trimethoprim and 100 mg sulfamethoxazole per Kg egg-weight dissolved in 0.3 ml phosphate buffered saline solution on the 18th day of incubation through a 22mm needle. Second and third groups were maintained as a sham control (0.3 ml sterile phosphate buffered saline solution) and un-injected control, respectively. Embryos received treatment by direct injection into the yolk sac according to the standard techniques (Hamburger 1942; Tavakkoli et al. 2013). The in ovo injection was done through a pinhole made at the broad end of the egg and was completed within 15 minutes. Immediately after the injection, the site was sealed with sterile paraffin and eggs were re-incubated post-treatment and allowed to develop. The control group, which did not receive any injection was kept in an equal condition for 15 minutes to equate the injectable environment. Sterile phosphate buffered saline solution was included as a sham control to rule out a possible negative response caused by the stress of injection. The viability of the embryos was checked throughout the incubation period by candling. All embryos were necropsied on the 21th day of incubation and examined for macroscopic and microscopic lesions. The embryos were humanely killed by placing on ice and then the eggs were opened at the wider end (Jacobsen et al. 2012; Tavakkoli et al. 2014). 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 µm thicknesses were cut using a microtome (Slee-Germany) and stained with hemotoxylin and eosin and studied under light microscope. 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.

Statistical analysis

Statistical analysis was performed using SPSS version 20. The Chi-Squar 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

Gross evaluation

The tissues of the embryos, such as the skin, brain, heart, muscle, liver, kidney and lung were normal in the sham control (0.3 ml phosphate buffered saline solution) and un-injected control groups. In the trimethoprim/sulfamethoxazole-injected group, group 1, there was not any gross abnormality in the internal tissues and external body surfaces. The obtained tissue samples of these embryos were sent to the pathology laboratory.

Histopathological evaluation

Histopathological evaluation has been revealed that all organs were normal in the sham control and un-injected control groups. In the embryos of group 1, which received the trimethoprim/sulfamethoxazole injectable solution, all microscopic structures were also normal. The photomicrograph of the skin, brain, muscle, liver and lung tissues were demonstrated in figures 1 to 5. No histopathological alterations are seen in the above mentioned tissues.

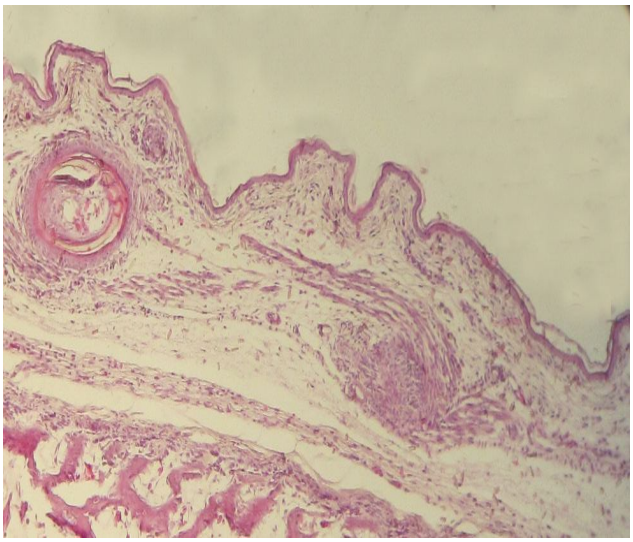

Fig. 1. Photomicrograph of the partridge embryo treated with trimethoprim/sulfamethoxazole injectable solution into the yolk sac. A normal structure of the skin is seen. ×100 H&E

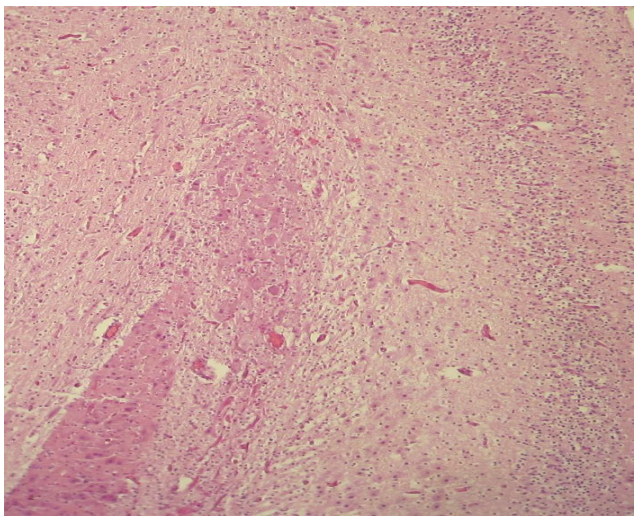

Fig. 2. Photomicrograph of the partridge embryo treated with trimethoprim/sulfamethoxazole injectable solution into the yolk sac. A normal structure of the cerebrum is seen. ×100 H&E

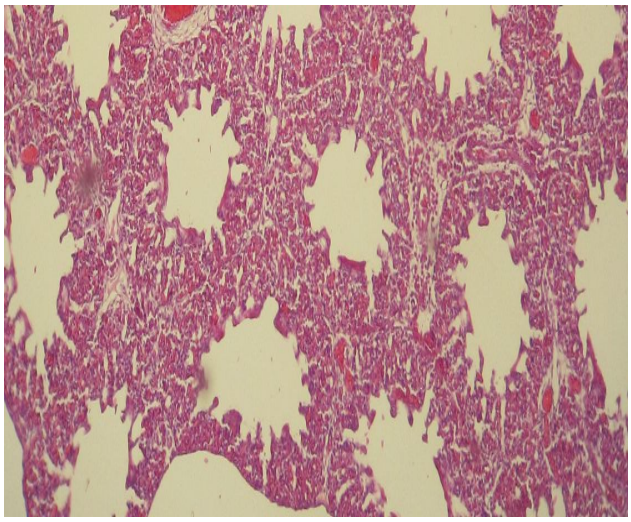

Fig. 3. Photomicrograph of the partridge embryo treated with trimethoprim/sulfamethoxazole injectable solution into the yolk sac. The normal structure of the lung is seen. ×100 H&E

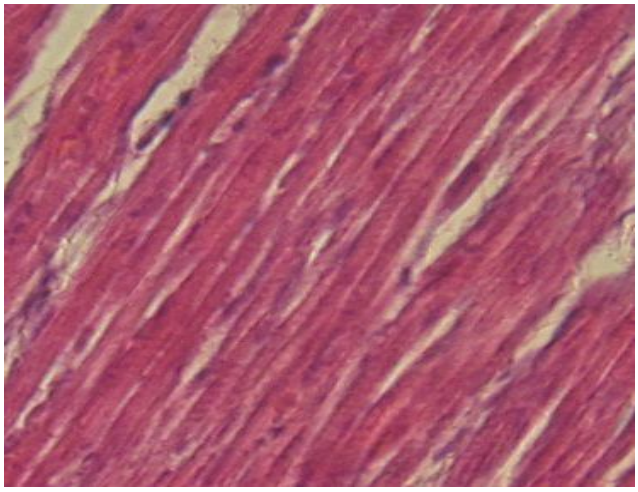

Fig. 4. Photomicrograph of the partridge embryo treated with trimethoprim/sulfamethoxazole injectable solution into the yolk sac. The normal structure of the muscle is seen. ×400 H&E

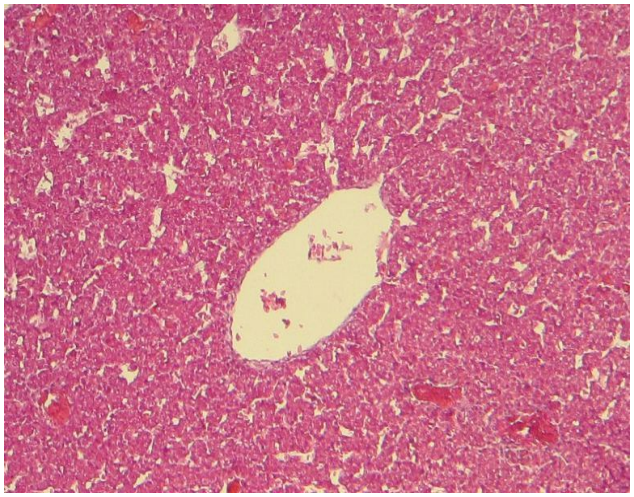

Fig. 5. Photomicrograph of the partridge embryo treated with trimethoprim/sulfamethoxazole injectable solution into the yolk sac. The normal structure of the liver is seen. ×100 H&E

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 (Colomer-Lluch et al. 2011; Sapkota et al. 2011; Obeng et al. 2012; Banerjee et al. 2013; Tavakkoli et al. 2104), quail (McDougald et al. 2012; Crespo et al. 2013; Rigobelo et al. 2013), turkey (Altunsoy et al. 2011; Erdem and Akova 2012; Buscaglia 2013), broiler (MacDonald and Wang 2011; Agunos et al. 2012; Lee et al. 2012), layers (Hasan et al. 2011; Lee et al. 2013; Nemati 2013) and poultry breeder (Kabir 2010; Priyantha et al. 2012; Jones et al. 2013).

Folate antimetabolite/sulfonamide drugs have an increased role as therapeutic agents against avian pathogens. They have a wide antibacterial spectrum. Most gram-positive and gram-negative organisms are susceptible (Sweetman et al. 2009; Ahrens and Martin 2013). Trimethoprim/sulfamethoxazole belongs to the folate antimetabolite/sulfonamide 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 trimethoprim/sulfamethoxazole 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 trimethoprim/sulfamethoxazole 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 (Ghazikhanian et al. 1980; Sheeks and Sheeks 1992; Kleven 2008; Singroha et al. 2012; Singroha et al. 2013; Tavakkoli et al. 2014). 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). 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 (Nascimento et al. 2005). On the other hand, some injection sites that are present in fertile eggs 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 et al. 2005).

Our results obviously showed no gross abnormality in the tissues and external body surfaces of the partridge embryos exposed to trimethoprim/sulfamethoxazole 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 trimethoprim/sulfamethoxazole injection sites in ovo may be the yolk sac. Nevertheless, further efforts are needed to evaluate in ovo administration of various folate antimetabolite/sulfonamide drugs for prevention and eliminate pathogenic microorganisms.

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

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