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Efficacy of Hydrogen Peroxide and Dihydroxy Benzol Mixture (Disinfectant) on *Toxocara canis* Eggs

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ABSTRACT

The main goal in the current investigation was to test the effect of a commercial disinfectant, hydrogen peroxide 50% and dihydroxy benzol 100 ppm solution, on the vitality of *Toxocara canis* eggs aiming to avoid contamination in kennels and veterinarian clinics. The present study added a new advantage to the previously known disinfectant, moreover to the previous disinfection benefits of this mixture. It showed high inhibitory activity on vitality of unembryonated *T. canis* eggs which depended basically on the tested concentrations. The best effect was observed with 3% concentration after 24 h exposure, where the inhibitory activity was 99.73% and the egg development was arrested at two-cell stage. On the other hand, there were neither morphological changes nor any cessation of the motility of larvae in embryonated *T. canis* eggs, with all disinfectant concentrations. The present study supported the use of hydrogen peroxide 50% and dihydroxy benzol 100 ppm solution at 3% concentration as a disinfectant agent against *T. canis* eggs because of its ovicidal effect on unembryonated eggs.

Key words: *Toxocara canis*, disinfectant, hydrogen peroxide, ovicidal, efficacy

INTRODUCTION

Toxocara canis (Werner 1782) is a widespread nematode affecting pets like dogs and cats, especially puppies. Humans become infected by inadvertent ingestion of eggs containing second-stage *T. canis* larvae which may subsequently spread throughout the body (Minvielle *et al*., 1999). The major clinical consequences of prolonged migration of *T. canis* larvae in humans are visceral larva migrans and ocular toxocariasis (Glickman and Magnaval, 1993). There is a strong correlation between frequency of *Toxocara* infection, life style and infection risk. Toxocariasis is present worldwide but people living in areas, with sanitary deficiencies are considered at the highest risk of infection (Despommier, 2003). *T. canis* eggs passed in faeces are unembryonated and ineffective. These eggs are subglobose, 75×85 μm in size and have a thick pitted shell. When first passed, they are embryonated and must undergo further development in conditions of suitable temperature and humidity. The process takes 3-4 weeks and the eggs remain infective for a long period; many months or even years, because of their extremely resistance to chemical and climatic agents, becoming the source of infection to both animals and humans (Glickman and Schantz, 1981; Deutz *et al*., 2005). The elevated resistance of the *T. canis* eggs has stimulated research on

disinfection however, they could survive in 2% glutaraldehyde, 10% benzalkonium chloride, 7% sodium hypochlorite, 1% potassium permanganate, 70% ethyl alcohol, 10% potassium hydroxide and 3% phenol solutions (Aycicek *et al*., 2001). Moreover, they could embryonate successfully in 2% formalin, in potassium dichromate and in 50% solutions of hydrochloric, nitric, acetic and sulfuric acids (Marvin and Olsen, 1992).

In time, where numerous disinfectants were reported as unsuitable for *T. canis* eggs eradication, hydrogen peroxide (H_2O_2) is one of the most powerful commonly used oxidizers. It is very unstable and breaks down readily into water and a single oxygen molecule which is a strong oxidizing and disinfecting agent (Parkes and Mellor, 1947). Merck, s index indicated that hydrogen peroxide could be used as a water disinfectant. In the medical world it was used as a topical disinfectant. The tested disinfectant is a mixture of hydrogen peroxide 50% and dihydroxy benzol 100 ppm. It is a naturally powerful disinfectant contains free peroxide prolonged chain still effective up to 48 h. It is applied as an aqueous solution with the final concentrations of 0.003, 0.006, 3.0, 3.0 and 1.5% in drinking water of animal and human, chillers, poultry houses and hatching eggs, respectively.

The main goal in the current investigation was to test the effect of this known disinfectant on the vitality of *T. canis* eggs aiming to avoid contamination in kennels and veterinarian clinics.

MATERIALS AND METHODS

***T. canis* eggs:** Adult *T. canis* worms were collected from the intestines of naturally infected stray dogs that were killed by Egyptian police. The eggs were obtained from the uteri of adult female worms and stirred in a magnetic stirrer for 10 min with 1% sodium hypochlorite. This suspension was filtered through a sieve with 100 μ pores. The filtrate was centrifuged at 500×g for 3 min 3 times with 0.9% NaCl solution in order to remove sodium hypochlorite (Oshima, 1961). Number of unembryonated eggs mL^{-1} of solution was determined and kept in the refrigerator at 4°C until used.

Tested disinfectant: Mixture of hydrogen peroxide 50% and dihydroxy benzol 100 ppm, field available commercial product; sold as powerful disinfectant, was obtained from Alexandria for Chemical and Pesticides Co., Egypt. It was tested in five concentrations of 0.25, 0.50, 1.0, 2.0 and 3.0%. Each concentration was tested at 24 h exposure time according to the immersion technique of WHO (1961).

Efficacy of the tested solution on unembryonated *T. canis* eggs: For evaluation of efficacy of this product on unembryonated eggs, suitable medium sized Petri dishes; each contained 5000 eggs in a tested concentration of H_2O_2 and dihydroxy benzol in dublects at 24 h exposure time, as well as control eggs in distilled water were prepared. At the end of the exposure period, several times of washing and sedimentation using distilled water were carried out to get rid the remnant of the tested solution. The rate of embryonic development in the exposed eggs were evaluated after this, where the collected eggs were transferred into identified Petri dishes and incubated at 28°C for 3 weeks in 0.5% formalin (Oshima, 1961). The number of eggs showing embryonic development or second stage larvae (L2) in 10 randomized fields each of 100 eggs, in exposed as well as control eggs was counted. The reduction percentage of *T. canis* egg development induced by the tested solution was estimated using the following formula:

$$\text{Inhibitory activity} = \frac{\text{\% of control eggs containing L2 - \% of exposed eggs containing L2}}{\text{\% of control eggs containing L2}} \times 100$$

Efficacy of the tested solution on embryonated *T. canis* eggs: Second stage larvae containing eggs had been obtained after 3 weeks incubation at 28°C in 0.5% formalin. The embryonated egg suspension was centrifuged at 500×g for 3 min and washed 3 times in sterile distilled water in order to remove the formalin. The embryonated eggs were exposed to the tested disinfectant concentrations as before. The vitality of the exposed embryonated eggs was evaluated in terms of the motility of L2. The external chitinous layer of *Toxocara* eggs was removed by incubating the eggs in a 6% sterile sodium hypochlorite solution for 5 min at room temperature (Oshima, 1961). *T. canis* L2 vitality was evaluated by pressing the cover-slip and by analyzing the larval motility after increasing the temperature. The number of eggs showing motile L2 in 10 randomized fields, in exposed as well as control eggs was counted to determine the motile larvae percentage.

Statistical analysis: Data in Table 1 was statistically analyzed by ANOVA that was used to test for differences between the five concentrations of the tested disinfectant and control means and Duncan's test was used to separate means at stated level ($p<0.01$) using SPSS computer program.

RESULTS

Table 1 reflected that most of the *T. canis* eggs remained in developmental stages after incubation with 3.0% of the tested disinfectant (85.07%) and the percentage of eggs with developmental stages was significantly lower with the other concentrations of the tested disinfectant as well as in the control eggs. The highest inhibitory activity on egg development of the tested disinfectant reached to 99.73% with concentration of 3.0% and the egg development was arrested at two-cell stage (Fig.1d, e, f). At 2.0% concentration, low inhibitory activity was observed (14.01%) and small percentages of eggs remained in morula stage (Fig. 1g, h, i). No detectable

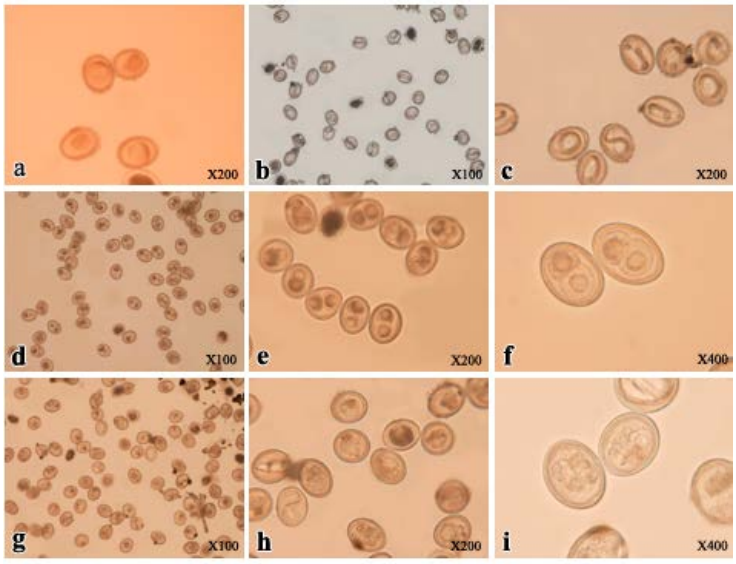

Fig. 1: *T. canis* eggs. (a) Unembryonated eggs. (b, c) Embryonated control eggs contained second larval stage. (d, e, f) After exposure to 3% concentration of the tested disinfectant. Note the egg development was arrested at two-cell stage. (g, h, i) After exposure to 2% concentration of the tested disinfectant. Note small numbers of eggs remained in morula stage

Table 1: Effect of hydrogen peroxide 50% and dihydroxy benzol 100 ppm solution on unembryonated *T. canis* eggs

	Mean number of *Toxocara canis* eggs* with			
Tested H_2O_2 concentration (%)	Zygote	Developmental stages	Second larvae (L2)	Inhibitory activity (%)
0.25	4.23±0.94^{b}	10.29±1.66^{c}	85.48±0.99^{d}	0.00
0.50	4.81±1.37^{b}	14.43±1.67^{bc}	80.76±0.60^{cd}	5.52
1	7.28±1.32^{b}	17.10±3.61^{b}	75.62±2.89^{bc}	11.53
2	8.37±2.30^{b}	18.14±2.04^{b}	73.50±1.81^{b}	14.01
3	14.71±1.30^{a}	85.07±1.13^{a}	0.23±0.23^{a}	99.73
Control	5.39±1.45^{b}	9.13±1.34^{c}	85.48±2.70^{d}	

* In 10 randomized fields each of 100 eggs. a, b, c means different concentration means within the same column are significant according to Duncan test $p<0.01$

inhibitory activity on egg development was recorded with the other concentrations as well as in the control eggs. On the other hand, in all disinfectant concentrations and in the control eggs, there were neither morphological changes nor any cessation of the motility of larvae in embryonated *T. canis* eggs.

DISCUSSION

T. canis has received a great deal of attention in face of its zoonotic potential (Robertson and Thompson, 2002) which is a direct consequence of soil contamination with feces of carrier animals as well as greater contact with dogs (Wolfe and Wright, 2003). Therefore, disinfection of kennels and veterinarian clinics, as a prophylactic measure, is getting more and more important. Most of cleaning in veterinary hospitals consists of disinfection which reduces the risk of infection from microbial contamination only. *T. canis* eggs, with the help of a strong shell structure, are very resistant to all environmental conditions and chemical agents and can survive outside of a host for approximately 6 years (Aycicek *et al*., 2001). Adult *T. canis* have a life span of about 4 months and females can produce 200,000 eggs per day. However, infection rates are much lower in adult dogs than in puppies and hence faecal shedding of eggs is much greater in pups than in adults. Puppies can shed 15,000 eggs per gram of faeces per day (Schantz and Stehr-Green, 1995).

The present study added a new advantage to the previously known disinfectant; hydrogen peroxide and dihydroxy benzole solution, moreover to the previous disinfection benefits of this mixture. It showed high inhibitory activity on vitality of unembryonated *T. canis* eggs which depended basically on the tested concentrations. The best effect was observed with 3% concentration after 24 h exposure, where the inhibitory activity was 99.73%. Very few chemical agents were effective against *T. canis* eggs. A 7% sodium hypochlorite was recommended as a disinfectant agent against *T. canis* eggs in textbooks and papers (Aycicek *et al*., 2001). On contrary, the same authors showed that the use of iodine solution was the best way to destroy *T. canis* eggs, whereas 7% sodium hypochlorite was ineffective. Hydrogen peroxide is an oxidizing agent close related to ozone that causes microbial death by protein denaturation (Gardner and Peel, 1986). As such, it found wide application for disinfection processes in the food, water treatment and healthcare. It had been reported to be effective in inactivating cysts of *Giardia lamblia* or *Cryptosporidium parvum* (Korich *et al*., 1990; Finch *et al*., 1993). Nevertheless, Ooi *et al*. (1998) showed that ozone alone had no apparent ovicidal and larvicidal effects on *T. canis*. The discrepancy in the effect of ozone stimulated the authors to speculate that if ozone in combination with other agents, be it chemical or physical which when exposed alone might not have an ovicidal

effect, might give rise to a strong synergistic ovicidal effect. Indeed, dihydroxy benzole in the tested disinfectant might improve the oxidizing action of hydrogen peroxide by forming hydroxyl radicals which were said to be the strongest oxidant known. It was generally considered that the inhibition of microbial growth by hydrogen peroxide was not the direct result of its oxidative properties in its molecular state but the consequence of the activity of other strongly oxidant chemical species derived from it. In fact, hydrogen peroxide is an excellent source of singlet oxygen, superoxide radicals and hydroxyl radicals that are highly reactive and very toxic for microorganisms (Halliwell and Gutteridge, 1984). Although the exact mechanism by which hydrogen peroxide produced lethal products for many microorganisms had not been clearly and completely elucidated, it was well known that, due to its ability to produce the above mentioned derivatives with strong oxidative properties, it could produce damage to nucleic acids, enzymes and membrane constituents (Schurman, 2001). However, it had also been reported that aqueous solutions of hydrogen peroxide alone would not cause protein, lipid, or nucleic acid modifications without the presence of catalysts for radical formation (Juven and Pierson, 1996). Moreover, advanced oxidizing processes; based on a combination of two oxidizing compounds, had been ever more widely employed for their higher stability and better results (Alam and Ohgaki, 2002; Wagner *et al*., 2002). The synergistic action of metal ions (silver and ferrous) and hydrogen peroxide had also been used for both drinking water and wastewater as well as cattle slurry disinfection (Hadziosmanovic *et al*., 1994; Tofant *et al*., 2001). In previous study, hydrogen peroxide 50% and dihydroxy benzol 100 ppm solution showed marked destructive effect on *Eimeria* spp. oocysts (Shalaby *et al*., 2004). It induced marked shrinkage of the oocysts' internal wall and structures. This was an advantage over numerous disinfectants included iodophor, sodium hypochlorite, formalin, copper sulphate, potassium hydroxide and potassium iodide at concentration of 4.0, 3.0, 5.0, 5.0 and 5.0%, respectively, as well as formaldehyde and peracetic acid via fumigation which were recorded by Campbell *et al*. (1982) as unsuitable for oocysts control. The superiority of hydrogen peroxide over other disinfectants in killing oocysts was related directly to its ability to penetrate the electron dense outer layer of the oocyst's wall. Indeed, hydrogen peroxide was not a large molecule and was able to diffuse through the cell membrane and, once inside, to produce hydroxyl radicals which might impact on different components of the cell producing the oxidative stress that led to irremediable consequences (Halliwell and Gutteridge, 1984). Nevertheless, the present study showed that the hydrogen peroxide 50% and dihydroxy benzol 100 ppm solution had no apparent larvicidal effect on *T. canis* as demonstrated by the lack of difference in larval motility between disinfectant-exposed and control eggs. The discrepancy in the effect of the tested disinfectant seen in the present study might be attributed to the fact that *T. canis* larva was basically different from a single cell embryo.

In the author's opinion, risks to human and animal infections can be more effectively and more practically reduced by maintaining dogs free of worms by regular administration of anthelminthics, removal of dog faeces from the environment before the eggs are able to embryonate (i.e., within 2 weeks), good personal hygiene and cleaning the veterinary clinics, hospitals, laboratories as well as animal shelters and cages with effective disinfectant. The present study supported the use of hydrogen peroxide 50% and dihydroxy benzol 100 ppm solution at 3% concentration as a disinfectant agent against *T. canis* eggs because of its ovicidal effect on unembryonated eggs. The natural extension of this work is to study the effect of this disinfectant on infectivity of embryonated eggs of *T. canis*.

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