In vitro EVALUATION OF THE DISINFECTION EFFICACY ON *Eimeria tenella* UNSPORULATED OOCYSTS ISOLATED FROM BROILERS

JOSÉ S. GUIMARÃES JUNIOR[1]; ALEXEY L. GOMEL BOGADO[2]; THIAGO CEZAR B. DA CUNHA[3]; JOÃO LUIS GARCIA[1]

ABSTRACT:- GUIMARÃES JUNIOR, J.S.; BOGADO, A.L.G.; CUNHA, T.C.B. DA; GARCIA, J.L. [***In vitro* evaluation of the disinfection efficacy on *Eimeria tenella* unsporulated oocysts isolated from broilers**]. Avaliação *in vitro* da eficácia de desinfecção sobre oocistos não esporulados de *Eimeria tenella* isolados de frangos de corte. *Revista Brasileira de Parasitologia Veterinária*, v. 16, n. 2, p. 67-71, 2007. Departamento de Medicina Veterinária Preventiva, Centro de Ciências Agrárias, Universidade Estadual de Londrina (UEL), Caixa Postal 6001, Londrina, PR 86051-970, Brazil. E-mail: jsgj@uel.br

The objective of this study was to evaluate *in vitro* the action of eight chemical principles by disinfection efficacy (DE) of *Eimeria tenella* oocysts. Disinfection efficacy was evaluated by either destruction or sporulation inhibition of the oocysts. Eight treatments were performed: T1 (Glutaraldehyde 42.5g + Benzalkonium Chloride 7.5g); T2 (Benzalkonium chloride + quaternary ammonium salt); T3 (formol 37% + Sodium Dodecylbenzene Sulfonate 12%); T4 (sodium hypochlorite 2%); T5 (Orthodichlorobenzene 60% + Xylene 30%); T6 (Polyoctyl polyamino ethyl glycine + Polyoxyethylene alkylphenol ether + Sodium Chloride); T7 (Chloramine T) and finally T8 (free iodine 2.25% + Phosphoric acid 15g). The control test was carried out with distilled water (T9). The best DE were observed, respectively, in T3 (79.49%), T5 (75.60%) and T4 (65.56%) treatments.

KEY WORDS: *E. tenella*, Oocysts, Disinfectant, Sporulation.

RESUMO

O estudo teve por objetivo avaliar, *in vitro*, a Eficácia de Desinfecção (ED) de oito princípios ativos sobre oocistos não esporulados de *Eimeria tenella*, por meio da capacidade de lise ou inibição da esporulação, submetidos à ação dos seguintes grupos de desinfetantes: T1 (glutaraldeído + cloreto de benzalcônio); T2 (cloreto de benzalcônio + sal quartenário de amônio); T3 (formol a 37% + dodecilbenzeno sulfonato de sódio 12%); T4 (hipoclorito de sódio 2%); T5 (ortodiclorobenzeno 60% + xilol 30%); T6 (polioctio-poliamino-etil-glicina + polioxi-etileno-alquil-fenol-eter + cloreto de sódio); T7 (cloramina T) e T8 (iodo 2,25% + ácido fosfórico). O controle (T9) foi realizado com água destilada. A maior ED atribuiu-se ao T3 (79,49%), seguido por T5 (75,59%) e T4 (65,56%).

PALAVRAS-CHAVE: *E. tenella*, Oocistos, Desinfetante, Esporulação.

INTRODUCTION

The cleaning procedure and disinfection of the installations, equipment and environment have fundamental importance in the sanitary management in broiler house. In the production cycle, between the check out of broiler flocks and the check in of another one, the disinfection followed by the sanitary depopulation is a necessary measure for the reduction of the infection pressure and the coccidiosis control, although some oocysts survive the procedure (VERMEULEN et al., 2001).

According to Daugschies et al. (2002), the objective of such conduct is to help the reduction of the exogenous *Eimeria* spp. stages, associated with the preventive use of anticoccidials. McDonnell and Russell (1999), and Williams (1997), do mention that protozoan oocysts are very resistant to most of the disinfectants. The first authors classify the oocysts as the most resistant etiological agents to the disinfectants, after the Prions. Sporulated oocysts are the infectant forms of *Eimeria* found in the environment. The sporulation depends on several environmental factors, such as: temperature, humidity and oxygen availability (FAYER, 1980) and on the parasite's characteristics, like disinfectants resistance and adverse field conditions that, associated,

[1] Departamento de Medicina Veterinária Preventiva, Centro de Ciências Agrárias(CCA), Universidade Estadual de Londrina (UEL), Caixa Postal 6001, Londrina, PR 86051-970. E-mail: jgsj@uel.br

[2] Curso de Pós-Graduação em Sanidade Animal, CCA,UEL.

[3] Curso de Medicina Veterinária da UEL, Londrina, PR.

contribute to coccidiosis remaining causing losses in poultry industry (PAGANINI et al., 2002).

After oocysts reach the environment, *Eimeria tenella* sporulation happens in at least 18 hours (REID et al., 1991) in broiler houses showing conditions for this event. This species is one of the most pathogenics; it has been responsible for economic losses in poultry industry worldwide (OUARZANE et al., 1998). The Disinfection Efficacy (DE) is defined as the sporulation inhibition percentage of oocysts (DAUGSCHIES et al., 2002). The cidal effect of the disinfectant is indicated by its effective action on not sporulated oocysts after in vitro incubation (WILLIAMS, 1997). This action is observed under two forms on the optic microscope: the oocysts present have lysed wall structure, therefore, non occurring sporulation; or the wall keeps itself visually preserved, however, the oocysts did not sporulate. Both ways are not infective. The complete oocysts lysis, a fact that may occur and is not visualized by optic microscope, can cause alterations in estimated ED values (DAUGSCHIES et al., 2002).

Most of the available products with disinfection action on oocysts are caustic or toxic, so their use in poultry installations is unsuitable (FAYER, 1980). Studies have been done worldwide to evaluate the action of different active principles with disinfectant action over *Eimeria* spp. oocysts (DI FÁBIO, 1994).

In Brazil, the Ministry of Agriculture, Livestock and Supply (Ministério da Agricultura, Pecuária e Abastecimento) is responsible for establishing the guidelines, liberation and inspection of disinfectants existent in the market for trading, through Decree nº 5.053 and Ordinance nº 193. According to it, "the disinfectant or antiseptic power must be evaluated by means of an adequate test recommended in recognized bibliography or developed methods and validated by the manufacturer", (BRASIL, 1998; BRASIL, 2004). This study had the objective of evaluating the disinfection efficacy of eight active principles on *E. tenella* oocysts.

MATERIAL AND METHODS

Hubbard chicken, with a day of age, under parasite free conditions, were provided with feed containing anticoccidial drug until 10 days old. From the 11th day of age on, the feed was free of anticoccidials drugs, maintained until the end of the study. The birds had access to water continuously. A total of 30 birds were inoculated in the 15th day, after fasting of 12 hours. Each bird received 11.6 x 10^4 sporulated *Eimeria tenella* oocysts of a field strain, by gavage. The feces were harvested and processed for eliminated oocysts quantification, using modified Gordon and Whitlock (1939) technique, six days post infection (dpi).

The disinfectants were diluted in 50 mL of solution containing distilled water according to the recommendation by the manufacturer for use in installations (Table 1) and distributed in Petri dishes. It was utilized a repetition for each one of the chemical agents and a control containing distilled water. The oocysts remained exposed to disinfectants for a period of 30 minutes. After this period, the oocysts were purified, meaning they were sedimented by centrifugation at 1300xG for eight minutes, being discarded the supernatant. Right away, they were diluted in distilled water one more time and centrifugated as described previously.

This trial was repeated from three to five times, for elimination of disinfectants residues. In sequence, the oocysts were put in Petri dishes containing potassium dichromate 2.5%

Table 1. Active principle, used dilution and the volume used in 50 mL of diluent (distilled water).

Nº	Active Principle	Dilution	Volume Used in 50 mL
T1	Glutaraldehyde 42.5g Benzalkonium Chloride 7.5g Alkyl Dimethyl Benzyl Ammonium Chloride	1:1000	0.05 mL
T2	(Benzalkonium chloride – quaternary ammonium salt) Formol 37%	1:200	0.25 mL
T3	Sodium Dodecylbenzene Sulfonate 12%	1:0	50.00 mL
T4	sodium hypochlorite 2% Orthodichlorobenzene 60%	1:0	50.00 mL
T5	Xylene 30% Emulsifier 10% Polyoctyl polyamino ethyl glycine 18.0 mL	1:200	0.25 mL
T6	Polyoxyethylene alkylphenol ether 12.0 mL Sodium Chloride 4.0 g Vehicle qs to 100 mL	1:1000	0.05 mL
T7	Chloramine T (n-chloro-para-toluene sulfonamide sodium salt) 99.6 g Excipient qs to 100 mL	1:100	0.5 g
T8	Iodophor Concentrated 11.25 g (contains 2,25 % free iodine) Phosphoric acid 15 g Vehicle qs to 100 mL	1:100	0.5 mL
T9	Control (distilled water)	1:0	50.00 mL

solution for preservation and it was maintained for a period of six days, under aeration, at room temperature varying from 25° to 30ºC. The oocysts were centrifuged once again to remove the potassium dichromate solution and the sediment dissolved in distilled water. For the counting, it was utilized standardized samples of 3 mL, this quantification was made by using Gordon and Whitlock (1939) modified method.

The Disinfection Efficacy (DE) was measured in percentage, and this represented the *in vitro* proportions of sporulated, and unsporulated or lysed oocysts (DAUGSCHIES et al., 2002). Differences among test proportions were compared by chi-square (χ^2) test with Yates correction, using EpiInfo 6.01 statistical package (DEAN et al. 1995). We have considered as significant a P value of ≤0.05.

RESULTS AND DISCUSSION

The statistical evaluations showed differences between the disinfectants regarding the oocysts sporulation inhibition capacity ($\chi^2 = 443.9$; P<0.00001, Table 2). This way, it can be concluded that the tested disinfectants formed two main groups (p<0.05): the first, composed of T3, T4 and T5, that showed a superior action, with a DE average of 79.5, 63.5, and 75.6%, respectively, and the second one T1, T2, T6, T7, and T8, which did not present efficiency in the oocysts sporulation inhibition. There was a statistical difference between T6 and T9 (control), however, the DE from that was lower than 10%.

Daugschies et al. (2002) evaluated cresol based disinfectants, among them the Preventol 4% and Neopredisan 3 and 4%. The DE obtained with Preventol 4% for the different strains was 17 and 49%. The authors observed that disinfectant efficiency was conditioned to the strain and to the exposition time of the oocysts to the disinfectant, determining that the disinfection success is directly related to the amount of time disinfectant are in contact with the oocysts.

Table 2. Sporulated and unsporulated oocysts by disinfectant action and the repetitions average.

Active principle	Rep	Oocysts (%)			DE(%)
		Sporulated	Unsporulated	Total	
T1	A	91.58	8.42	100	7.48[a,c]
	B	93.46	6.54	100	
T2	A	93.82	6.18	100	7.34[a,c]
	B	91.51	8.49	100	
T3	A	27.21	72.79	100	79.49[b]
	B	13.82	86.18	100	
T4	A	36.79	63.21	100	63.56[b]
	B	36.09	63.91	100	
T5	A	36.54	63.46	100	75.59[b]
	B	12.29	87.71	100	
T6	A	93.57	6.43	100	9.59[a]
	B	87.25	12.75	100	
T7	A	99.13	0.87	100	3.68[a,c]
	B	93.52	6.48	100	
T8	A	94.36	5.64	100	8.34[a,c]
	B	88.97	11.03	100	
T9	A	98.59	1.41	100	2.16[c]
	B	97.09	2.91	100	

($\chi^2 = 443.9$. P<0,00001)
Rep = Repetitions (A e B).
([a]),([b]) and ([c]) = Equal letter indicates no statistical difference (P>0.05), and unequal letter indicates statistical difference (P<0.05).

Senger (1959), in a study with oocysts of the *Eimeria bovis* species in USA, evaluated different active principles, obtaining ED percentages between 20 to 80%. Such study was delineated in a way to promote oocysts exposition to the active principle for 48 hours. This period represents a relatively long time, impratical on field, since many of those chemical agents are corrosives. It is desirable that the agents are rinsed quickly, increasing, this way, the lifespan of the broiler house equipment, despite these actions depend on the interaction with organic matter and its volatility (AYENE et al., 1972; WILLIAMS, 1997).

Williams (1997) tested the ability of two chemical agents to stop oocysts sporulation after in vitro incubation, phenol 41.6% and phenol 18.55% + chlorinated phenol 1.55%. The experiment was carried out in duplicate, with dilutions of 2.0; 1.0; 0.5; 0.25 and 0.125%. About the controls, the first one was composed of distilled water and the second one of a solution of ammonia 35%. The oocysts were exposed to the dilutions and controls during a period of 24 hours. The minimum effective concentration of the two principles and the control with ammonia 35% was between 0.4 and 0.5%, presenting, therefore, similar results in this concentration, according to statistical test carried out by the author. The ammonia 35% and the fenol 41.6% presented similar efficacies in low concentrations and showed up more efficient than phenol 18.55% + chlorinated phenol 1.55%.

The orthodichlorobenzene active principle evaluation, carried out by Nakano et al. (1971), achieved results of total destruction of the non sporulated form in oocysts of *Eimeria tenella* species, with the dilution of 1:400 from one minute on. This active principle corresponds to the disinfectant T5, also tested in this present work (75.79%), in a dilution of 1:200 during 30 minutes. The publication date of the referred article indicates more than 30 years between the studies. Therefore, certainly the lineages variations inside the *E. tenella* species and also the induced resistance of the inadequate management of disinfectants and anticoccidials drugs, and influenced by the results between the studies did not present similarity.

Oliveira et al. (2004) has tested the sporulation inhibition efficacy in oocysts of the *E. tenella, E. acervulina* and *E. maxima* species. A solution with mixed species was treated with commercial use disinfectants, and the exposition time was also of 30 minutes. The chemical agents were tested corresponding to this study, T1, T2, T3, T5 and T8. And the results were respectively: 11.0; 6.30; 25.0; 16.0; 7.8 and 5.5%. The active principles: formol solution 37% and phenol 10.5% + cresol 10.5%, were tested too, resulting in DE 43.0 and 7.7%, respectively. The desinfectants T3 (formol solution 37% + sodium dodecylbenzene sulfonate 12%) and T5 (orthodichlorobenzene 60% + xylene 30%) presented large differences in the results between the studies, with a high

variation coefficient of 73.75 and 92.01% respectively. There was not, therefore, a tendency to obtain similar results with these two chemical agents between the studies. The other disinfectants, including the control, showed up a regularity in the results, with a low variation coefficient.

The work of Hilbrich (1975) evaluated, in the period of 2, 10 and 30 minutes, the products Lysococ 5%; Pantek 2%; TGV2 2%; Incidin Anticoc 6%; NH4OH 4, 3, 2 and 1%; NaOH 10% and KOH 10% in oocysts not sporulated. In the results, were qualified as efficient the products Lysococ 5% and NH4OH 4%, presenting 100% of ED. The other ones were not efficient, with oocysts sporulation varying from 87 to 93%. The control presented 93% of sporulated oocysts. Right away, the oocysts were inoculated in birds and the products Lysococ 5% and NH4OH 4% were efficient again. There was no mortality of the birds and neither caecal lesions.

Barutzki et al. (1981) tested the disinfection efficiency of the products Lysococ 5 and 7.5%; Dekaseptol 6 and 9%; Lomasept 5 and 7.5% and Incicoc 5 and 7.5%, evaluated in sporulated oocysts of the *E. tenella* species. The authors observed that all the non sporulated oocysts of these treatments, were sensible to the disinfectants in the diverse concentrations, in a period from 2 to 20 minutes.

Ayeni et al. (1972), evaluating the action of 11 disinfectants on rodent oocysts, obtained a DE above 95%, in a period of five minutes, with three of the tested chemical agents: ammonia 3.7%; Dekaseptol 6% (carbon disulphide); Triseptol 5-6% (carbon disulphide, cresol, chlorinated cresols, chlorinated hydrocarbons). Knowing this disinfectant has different levels of reduction of its efficacy in the presence of organic matter, it can be concluded that the remaining organic matter after cleaning the poultry house, will be able to interact with the active principle used, causing alteration of its disinfection power (AYENI et al., 1972; WILLIAMS, 1997). The percentage of non sporulated oocysts would be able to increase in consequence of a large exposition to the disinfectant, when the test is done with different exposition times, according to Daugschies et al. (2002) and most of the studies mentioned demonstrated it. It is important that the same active principle presents action regularity in the different laboratories where it is tested (DAUGSCHIES et al., 2002). However, there is lack of stardandization, as much in the methodology as in the results' divulgation, where many times the numbers are not shown, causing difficulties in carried out studies' confrontations and showing contradictory results between laboratories that test the same chemichal agent (AYENI et al., 1972).

CONCLUSION

In the conditions that this study was carried out, it was observed the biggest ED for T3 (formol solution 37% + sodium dodecylbenzene sulfonate 12%) with 79.49%, followed by T5 (orthodichlorobenzene 60% + xylene 30%) presenting 75.59% and T4 (sodium hypochlorite 2%) with 65.56%. Such active principles presented, statistically, similar action in the oocysts sporulation inhibition of the *E. tenella* species.

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