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National Animal Production Research Institute
Ahmadu Bello University
P.M.B 1096, Shika-Zaria,
Kaduna State, Nigeria.
Email:japr@napri.gov.ng Url:journals.napri.gov.ng

OOCYSTICIDAL EFFECTS OF SOME COMMERCIAL POULTRY HOUSE DISINFECTANTS AGAINST SPORULATED *EIMERIA TENELLA* OOCYSTS

***[1]Jatau, I. D., [1]Aremu, S. F., [1]Magaji, Y. and [2]Babashani, M.**
[1]Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria.
[2]Veterinary Teaching Hospital, Ahmadu Bello University, Zaria.
**Corresponding author: E-mail: mail4idjatau@gmail.com, Tel.: +2348030569756.*

ABSTRACT

This study investigated the oocysticidal efficacies (OE) of some disinfectants (DIs) used in poultry house sanitations against sporulated *Eimeria tenella* oocyts (SEtO). About 2×10^3 SEtO were dispensed into each of a set of duplicate tubes (labelled 1 and 2) with each pair containing solutions of twice and four times the manufacturers recommended disinfection concentrations (MRDC) of Sodium hypochlorite (A); Saponated cresol (B); Cresol (C) and Formaldehyde solution (D) respectively, and allowed for 45 minutes on laboratory bench. The oocysts were washed off from the DIs and examined under the microscope to determine for the OE (percentage of lysed to normal SEtO) of each DIs. At concentrations twice the MRDC, all the tested DIs had low OE of 20.4±0.80%, 32.3±1.70%, and 20.8±1.20% respectively for DIs A, B and C. The disinfectant D had significantly ($P < 0.05$) lower OE of 14.3±0.70%. Increasing the concentrations to four times the MRDC significantly ($P < 0.05$) improved the OE to 39.0±3.0%, 50.2±3.80%, 59.9±0.70% and 30.3±1.70% respectively for DIs A, B, C and D with the disinfectant C having significantly ($P < 0.05$) higher OE. It is concluded that, while SEtO is considered resistant to disinfection, considerable increase of the concentration of cresol based DIs, effectively improve its OE against SEtO.

Key words: *Eimeria tenella*, sporulated oocysts, disinfectants, Nigerian poultry

INTRODUCTION

Avian coccidiosis is an economically important enteric protozoan disease of poultry worldwide; causing high morbidity and moderate mortalities rates (Shirley *et al*., 2005). *Eimeria tenella* considered as the most pathogenic specie in chickens, is the major cause of outbreaks in poultry farms due to its great potential to cause injury to birds, even with low dose of oocysts (Conway *et al*., 1993; Jatau *et al.*, 2014). Transmission of *Eimeria* parasite usually occurs via the ingestion of sporulated oocysts from the environment by a susceptible chicken (Shirley *et al*., 2005). The wall of *Eimeria* oocysts provides an effective protective barrier against the extremes of environmental conditions, mechanical disruption and chemical disinfectants (Belli *et al.*, 2006; Jatau *et al.*, 2013). Because of these characteristics, *Eimeria* oocysts can persist for several months in the environment and be spread to poultry houses mechanically by many routes such as personnel boots, cloths, wheels, contaminated equipment and dust (Taylor *et al*., 2007). *Eimeria* parasites have been reported in almost every poultry establishment worldwide (Daugschies *et al*., 2002; Clerck *et al*., 2016). This ubiquitous nature of the chicken *Eimeria* has precludes eradication as practical way of controlling coccidiosis especially in birds kept under deep litter system.

Current control of coccidiosis is primarily based on the use of anticoccidial drug (Taylor *et al*., 2007). The cost of chemotherapeutic control of coccidiosis makes it one of the most expensive parasitic diseases in the poultry industry (Bennett and Ijpelaar, 2005). The cheapest alternative to chemotherapeutic treatment of coccidiosis would be reduction of

infection pressure by early disinfection of the poultry facilities, poultry house and environment in the intervals between depopulation and restocking using chemicals that are established to be effective against the environmentally resilient sporulated oocysts. Preliminary study by Jatau *et al.* (2013) shows that cresol and sodium hypochlorite based disinfectants are effective against unsporulated *Eimeria tenella* oocysts, but not effective on sporulated oocysts, even at twice the manufacturer's recommended concentrations. Here we investigated the effects of Cresol, Sodium hypochlorite and saponated cresol based disinfectants as well as Formaldehyde on the sporulated oocysts of *Eimeria tenella* at concentrations of twice and four times the manufacturer's recommendations.

MATERIALS AND METHODS

Study Area

The study was conducted in the Department of Veterinary Parasitology and Entomology Faculty of Veterinary Medicine Ahmadu Bello University, Zaria. Zaria is located between latitude 11^0 07'N and longitude 7^0 44'E within the Northern guinea savanna zone. It possesses a tropical continental climate with a pronounced dry season, lasting up to seven months (October - May). The rainy season lasts from May to September/October. The average rainfall ranges from 1000 – 1250 mm and the average daily temperature ranges from 19 ^{0}C – 33 ^{0}C.

Isolation *of Eimeria tenella* Oocysts.

The *Eimeria tenella* stock used in this study was first isolated from the field and characterized by Jatau *et al*. (2016). The parasite was maintained in the laboratory at 4 °C and occasional passage in broiler chickens. The *Eimeria tenella* oocysts were propagated *in vivo* by oral inoculation of 1, 000 sporulated oocysts each to five 2-weeks old coccidia free broiler chicks. The progeny oocysts were then recovered from the caecal tissue collected during post-mortem 7 days post infection, sporulated in 2.5 % (w/v) potassium dichromate solution and purified as described by Eckert, (1995).

Chemical Disinfectants Used and their Reconstitutions

The disinfectants used in this study were Sodium hypochlorite 3.85% m/v, Saponated cresol, Cresol and Formaldehyde solution 40% w/v. Each of the chemical was reconstituted with distilled water to obtain twice and four times the manufacturers recommended concentrations as follows: 1:20 and 1:10 solutions for sodium hypochlorite; 1:49 and 1:24 solutions for saponated cresol; 1:49 and 1:24 solutions for Cresol; 10% and 20% solutions for Formaldehyde.

In vitro Exposure of Sporulated *Eimeria tenella* Oocysts to the Disinfectants

The *In vitro* exposure of the oocysts to the disinfectants was done in two phases. In the first phase, 9 ml solutions of twice the manufacturer's recommended disinfectant concentration of each of the chemicals was dispensed in duplicates test tubes viz: tubes A1 and A2; B1and B2; C1 and C2; D1 and D2 for sodium hypochlorite, saponated cresol, cresol and formaldehyde respectively. Test tubes E1 and E2 containing 9 ml of distilled water each served as the control. One millilitre of sporulated *Eimeria tenella* oocysts suspension containing approximately 2 x10^3 sporulated oocysts was dispensed in each of the test tube pairs A- E. The experimental set up were allowed to stay for 45 minutes on the laboratory bench after which the oocysts were washed off from the disinfectants by filling the tubes with distilled water and centrifugation at 300 × *g* for 5 min 3 times. The sediment of the final washing process containing the oocysts was used for the determination of the disinfectants efficacies.

In the second phase of the experiment 9 ml solutions of four times the manufacturer's recommended disinfectant concentration of each of the chemicals was used to exposed fresh portion of the sporulated oocysts suspension utilizing similar experimental set up and procedures as described in the phase I.

Determination of Oocysticidal Efficacy (OE) of the Disinfectants on the Exposed Sporulated *E. tenella* Oocysts

After the experimental exposure of the oocysts to disinfectants, the oocysticidal efficacy (OE) of each disinfectant was determined as follows: The sediment of the final washing process containing the oocysts from each test tube were view by direct smear of the oocysts suspension and examined under the microscope at x 400 magnification. Five hundred oocysts were counted in each slide examined with the aid of a blood cell counter (Clay Adams Inc. USA) to determine the proportion in percentage of lysed and or deformed oocysts to unlysed normal sporulated oocysts as described by (Daushies *et al*., 2002).

Data Analysis

Mean values of the OE were compared for the different concentrations of individual disinfectant and among the disinfectants using student t-test and analysis of variance (ANOVA) respectively using GraphPad prism. Values of $P< 0.05$ were considered statistically significant.

RESULTS

The result of *in vitro* oocysticidal efficacies (OE) of twice and four times the manufacturer's recommended disinfectant concentrations of sodium hypochlorite (A), Saponated cresol (B), Cresol (C) and Formaldehyde (D) solutions against sporulated *Eimeria tenella* oocysts is shown in table 1. At concentrations twice the manufacturer's recommended disinfectant concentrations, all the tested disinfectants had low OE of 20.4±0.80%, 32.3±17%, and 20.8±1.20% respectively for disinfectants A, B and C. The disinfectant D had significantly ($P < 0.05$) lower OE of 14.3±0.70% against the sporulated *Eimeria tenella* oocysts compared to the other disinfectants. Increasing the concentrations of the disinfectants by two folds (four times the manufacturer's recommended disinfectant concentrations) significantly ($P < 0.05$) improved the OE of all the tested disinfectants to 39.0±3.00%, 50.2±3.80%, 59.9±0.70% and 30.3±1.70% respectively for disinfectants A, B, C and D. The disinfectant C had significantly ($P < 0.05$) higher OE value compared to the others tested at the four fold concentrations.

Table 1: Oocysticidal efficacies (OE) of some disinfectants against sporulated *Eimeria tenella* oocysts

Active Principle	Dilution	Exp. Set- up	Number of Oocysts examined		OE (%) (Means ± SEM)
			Lysed	Unlysed	
A	1:20	A_1	98	402	20.4 ± 0.80^{a}
		A_2	106	394	
	1:10	A_3	210	290	39.0 ± 3.00^{b}
		A_4	180	320	
B	1:49	B_1	170	325	32.3 ± 1.70^{a}
		B_2	153	347	
	1:24	B_3	270	230	50.2 ± 3.80^{b}
		B_4	232	268	
C	1:49	C_1	110	390	20.8 ± 1.20^{a}
		C_2	98	402	
	1:24				
		C_3	302	198	$59.9 \pm 0.70^{**b}$
		C_4	297	203	
D	10%	D_1	75	425	$14.3 \pm 0.70^{*a}$
		D_2	68	432	
	20%	D_3	143	357	$30.3 \pm 1.70^{**b}$
		D_2	160	340	
E	-	E_1	2	498	$0.6 \pm 0.70^{*}$
		E_2	4	496	

A= Sodium hypochlorite, B = Saponated cresol, C = Cresol, D = Formaldehyde and E = Distilled water. SEM- Standard Error of Means. [a,b] OE values in the same column with different superscripts are significantly different for individual disinfectants using t-test ($p < 0.05$). *** OE values are significantly different from those without asterisk between different disinfectants (i.e. for A, B, C, D) using One-Way ANOVA ($p < 0.05$).

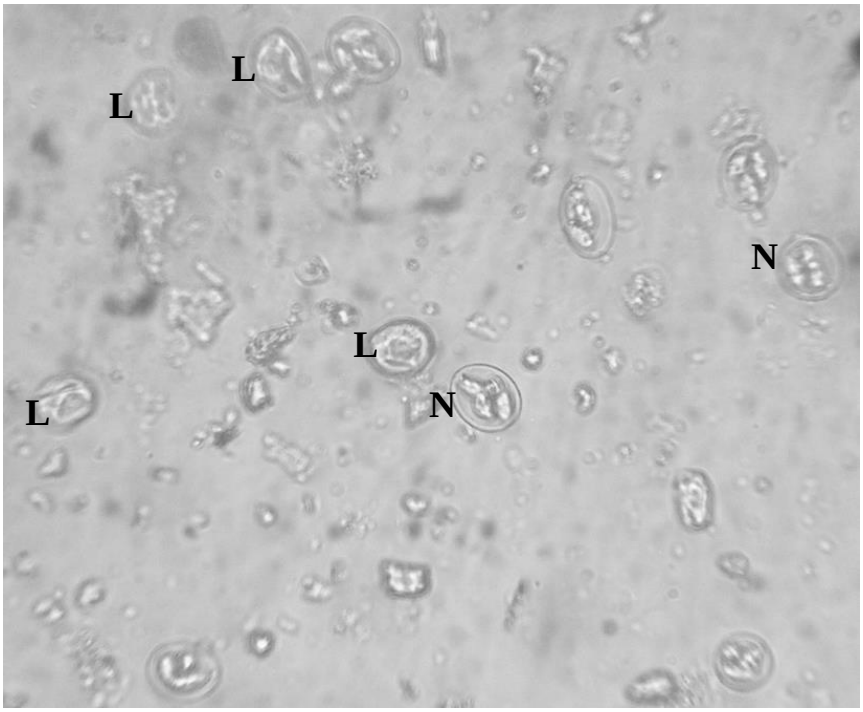


Figure 1: Disinfectant exposed sporulated *Eimeria tenella* oocysts. Note the lysed (L) and Normal (N) sporulated oocysts. (× 400)

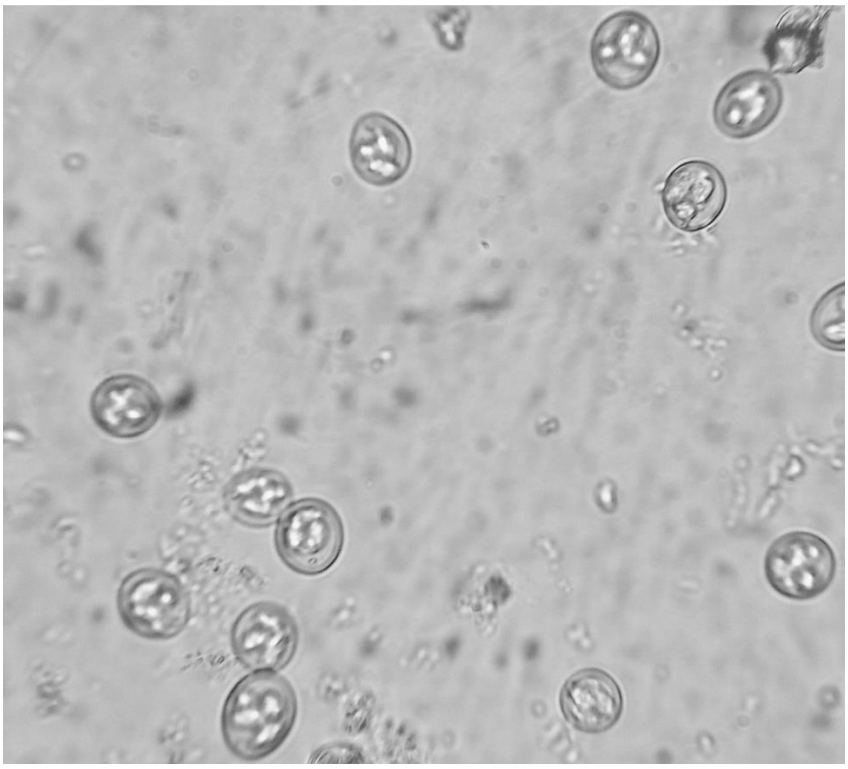


Figure 2: Sporulated *Eimeria tenella* oocysts exposed to distilled water (control) showing normal sporulated oocysts. (× 400)

DISCUSSION

The purpose of disinfection of poultry houses before re-stocking is to destroy viral and bacterial pathogens and exogenous forms of parasite. While many chemical disinfectants are effective against most bacterial and viral pathogens, coccidial oocysts are very resistant to physical and chemical treatment because of the two chemically distinct layers of combined lipids and glycoproteins that provides efficient protection of its germinal substance (Hi1brich 1975; Daugschies *et al*., 2013). Studies have shown that the unsporulated oocysts of *Eimeria* and *Isospora* may be vulnerable to the action of some disinfectants (Jatau *et al*., 2013; You, 2014) but, the sporulated oocyst have been shown to resist the effect of such disinfectants even at concentrations higher than the effective concentrations used against the unsporulated oocysts (Jatau *et al*., 2013). Because of their resilience, sporulated oocysts are the most commonly seen contaminants in infected poultry houses and initiates outbreaks in susceptible flocks. Here we investigated the cidal effect of some common disinfectants used in cleaning poultry houses in Nigeria against sporulated *Eimeria tenella* oocyts. All the disinfectants used had very low disinfectant efficacy against the sporulated *E tenella* oocysts at concentration twice the manufacturer's recommended disinfection concentrations a finding that corroborate previous reports that sporulated *Eimeria* oocysts are highly resistant to many disinfectants (Belli *et al*., 2006; Guimaraes *et al*., 2007). The dityrosine crosslinking lipoproteinous walls of the oocyst that subsequently become dehydrated ("tanned") and hardened are responsible for the notorious resilience of oocysts (Belli *et al*., 2006). The composition of these lipoproteins in the walls of sporulated oocysts are substantially higher than that of the unsporulated oocysts of *Eimeria* (Ferguson *et al*., 2003), thereby making the former more resistant to even the most aggressive disinfectants than the later. The cresol and saponated cresol based disinfectants appear to have better oocysticidal effects among the disinfectants used in the present work and agrees with the reports of Barutzki, *et al*., (1981) and Straberg and Daugschies (2007) which show that some significant level of disinfectant effects on the sporulated oocysts can be achieved by lipid dissolving substances such as cresol based products. Previous works have shown that disinfectant effects against coccidia oocyst is directly proportional to the concentration of its active ingredients (Chroustova and Pinka, 1987; Jatau *et al*., 2013; Guimaraes *et al*., 2007). In the present study we also observed significant increase in oocysticidal effects of all the disinfectants against the *E. tenella* oocyst when the concentrations were increased by two fold (four time the manufacturer's recommended concentrations) with the cresol based disinfectant having up to 60% oocysticidal effect. This suggests that at concentrations significantly higher than the concentrations used against other pathogens, cresol based disinfectants may be effective in reducing the risk of outbreak of coccidiosis in commercial poultry establishments. However, the biological hazard of the use of the disinfectants at such higher concentration need to be investigated to ascertain the safety of it's used in cleaning poultry houses or facilities.

Disinfectants effects against coccidia oocysts also depends on the length of time of exposure of the oocysts to the disinfectants (Chroustova and Pinka, 1987; Williams, 1997; You, 2014). Under practical condition disinfectants can be sprayed or poured onto poultry house surfaces and, after a brief period, cleaned up with towels or washed up with water. Effective disinfectants should kill target organism within short period of time. Incubations of disinfectants on spilled microorganisms longer than an hour may be impractical and unreasonable. The fact that the cresol based disinfectant in this study destroyed 60% of the sporulated oocysts within the 45 min period of exposure further suggests that it may be an effective alternative for the prevention of outbreak of coccidiosis.

Although only the oocysts of the most economically important *E. tenella* oocysts were used in the present study, we believe that the results may apply to the oocysts of the other poultry coccidia too, since the walls of oocysts of different species of chicken *Eimeria* have similar physical and chemical characteristics (Belli *et al*., 2006; Ferguson *et al*., 2003).

Results from previous works had shown that sporozoites may survive within a morphologically aberrant or normal oocysts after exposure to some disinfectants and thereby could be infective to susceptible hosts (Straberg and Daugschies, 2007; Jatau *et al*., 2013) which suggests that disinfection alone may not be solely relied upon in the prevention of outbreaks of coccidiosis in poultry farms. However, in poultry houses especially those with persistent outbreak of coccidiosis, physical measures (heat or drying) being not practicable in large poultry units, chemical disinfection is the only practical way to destroy the exogenous coccidia forms and should therefore be considered with same importance as medication with anticoccidial drugs in prevention of coccidiosis.

CONCLUSIONS

While sporulated *Eimeria* oocyst is considered highly resistant to disinfection, the present study has shown that increasing the concentration of cresol based disinfectants considerably, may significantly improve the cidal effect of the disinfectants against the oocysts.

We therefore, suggests that for a cheaper effective prevention of outbreak of avian coccidiosis, chemical disinfection of poultry house facilities should be combined with good management practices such as proper biosecurity measures, good sanitary measures and good litter management.

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CONFLICT OF INTERESTS

The authors have no conflict of interests to declare

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