Sanitary depopulation is a fundamental measure by which infection burden can be reduced, and thus could contribute to an effective coccidiosis control strategy. This strategy helps to reduce exogenous *Eimeria* spp. at different stages of development [1]. Coccidial oocysts are extremely resistant to physical and chemical stress [2,3]. Moreover, no internationally-accepted standardized method for testing antiparasitic properties of chemical disinfectants currently exists. Therefore, such a method needs to be developed in order to effectively and safely control coccidiosis in chickens [4-6].

Internationally there are no accepted standardized methods for testing antiparasitic properties of chemical disinfectants, although various models and procedures have been developed and are applied in many laboratories worldwide. Guimarase et al. [7] studied disinfectant effects (DEs) on *Eimeria tenella*, and in which 6 different disinfectants with different concentrations were used. The aim of the present study was to investigate the efficacy of various common disinfectants in reducing the viability of *E. tenella* oocysts specific to Korean field isolates.

Parasites were collected from infected commercial broiler chickens. After collection from broiler chickens, oocysts were purified by sedimentation method (1,300 g, 8 min). After discarding supernatants, the resultant sediment was diluted in distilled water and centrifuged once more as before. For oocyst enumeration, the MacMaster egg counting method was used. Correct species assignments and sample purities were confirmed by singular internal transcribed spacer (ITS)-PCR analysis. A total of 18 treatments were performed, and the disinfection suppression levels were 75.9% for 39% benzene + 22% xylene (1:10 dilution), 85.5% for 30% cresol soup (1:1 dilution), and 91.7% for 99.9% acetic acid (1:2 dilution) group. The results indicate that acetic acid, cresol soup, and benzene+xylene are good candidates for suppression of *E. tenella* oocyst sporulation.

**Key words:** *Eimeria tenella*, disinfectant, suppression, sporulation
- Cysts were then exposed to disinfectants for 30 min. The disinfectants used are shown in Table 1. The disinfectants used can be broadly divided into the following 10 groups: A1, 30% cresol soup (Chongsol Chemical, Seoul, Korea); A2, 39% benzene (Daejin Chemical, Seoul, Korea)+22% xylene (Daejung Chemical and Metal, Seoul, Korea); B1, 99.9% sodium hypochlorite (Yuhanclorox, Seoul, Korea); B2, 5% quaternary ammonium salt (Kwangjin Pharma, Seoul, Korea); B3, 6% quaternary ammonium salt+13% aldehyde (KBNP Inc., Seoul, Korea); B4, 10% malic acid (Wako Pure Chemical Industries, Osaka, Japan); B5, 88% carbamate type (Bayer Animal Health, Tokyo, Japan); C1, hydrogen peroxide (Wako Pure Chemical Industries); C2, 99.9% acetic acid (Wako Pure Chemical Industries); and C3, sodium hydroxide (Wako Pure Chemical Industries). Either 3 or 4 independent replicates were performed for each disinfectant. DEs were calculated according to the proportion of in vitro sporulated vs unsporulated or lysed oocysts [1].

The disinfectants tested ranged considerably in their capacities to inhibit oocyst sporulation (Fig. 1A-C). Out of the 3 main groups, A1 (1:1 dilution), A2 (1:10 dilution), and C2 (1:2 dilution) showed superior disinfection action, with effect rates of 85.5%, 75.9%, and 91.7%, respectively (Fig. 2). In comparison, all other groups exhibited much lower DEs, with the exception of the quaternary ammonium compound (1:5 dilution). Multiple dilutions were tested for cresol soup, benzene+xylene, quaternary ammonium salts, carbamate and acetic acid, since these substances are commonly used as disinfectants. Among these substances, cresol soup, benzene+xylene, quaternary salts, and acetic acid all inhibited *Eimeria* sporulation when used at increased concentrations.

**Table 1.** Chemical agents, recommended dilutions, and dilutions used

| Item | Chemical agents | Recommended dilution | Used dilution |
|------|-----------------|----------------------|--------------|
| A1   | Cresol soup 30% | 1:100                | 1:100, 1:10, 1:5, 1:1 |
| A2   | Benzene 39%+Xylene 22% | 1:500 | 1:1,000, 1:10, 1:10 |
| B1   | Sodium hypochlorite 99.9% | 1:10 | 1:10 |
| B2   | Quaternary ammonium salt 5% | 1:500 | 1:50, 1:5 |
| B3   | Quaternary ammonium salt 6%+Aldehyde 13% | 1:100 | 1:10 |
| B4   | Acids (malic acid) 10% | 1:200 | 1:10 |
| B5   | Carbamate type 88% | 1:25 | 1:88, 1:8 |
| C1   | Hydrogen peroxide 3% | 1:10 | 1:01 |
| C2   | Acetic acid 99.9% | - | 1:10, 1:2 |
| C3   | Sodium hydroxide (10N) | - | 1:01 |
| Con  | Control (distilled water) | | |

**Fig. 1.** Degree of sporulation of *Eimeria tenella* oocysts. (A) Nonsporulated oocyst (×1,000). (B) Sporulating oocyst (×1,000). (C) Sporulated oocyst (×1,000). Bar = 5 µm.
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The DEs of cresol-based disinfectants, such as Preventol (4%), were previously shown to range from 17% to 49% for various strains of coccidia [1]. These results are not in agreement with our findings, since it was observed that cresol effectively inhibited sporulation up to 85.5%. The cresol-based product, Neopredisan 135-1 (R) (NP), was previously shown to inactivate *Isospora suis* in vitro, with a concentration of 2-4% able to induce lysis of more than 95% of sporulated oocysts within a contact time of 30 min [9]. Furthermore, these conditions completely destroyed all oocysts after a contact time of 90 min or more [9]. These results were similar to the results observed for cresol. In another study, the ability of 11 disinfectants to inactivate oocysts in rodents was examined, with an efficacy greater than 95% obtained for 3.7% ammonia in a period of 5 min [10]. This result contradicts the data obtained in our study.

The anticoccidial effects of acetic acid at different concentrations in broiler chickens have also been previously evaluated and compared with those of amprolium; it was found that 3% acetic acid had the maximum anticoccidial effects [11]. This study also found that the maximum disinfecting effects were exerted by acetic acid, although this study used 99.95% at a 1:2 dilution. The antimicrobial effects of quaternary salts have also been tested, but none were shown to be effective against either the parasitic protozoan, *E. tenella*, or the helminth, trichostrongyle nematodes [5]. The present study found that quaternary salts only inhibited sporulation by 13%, even when used at a 10-fold greater concentration than the recommended dose. This study did not observe any disinfectant advantages of using a quaternary ammonium compound, even when it was mixed with aldehyde. Anticoccidial activity of herbal complex was also studied in broiler chickens challenged with *Eimeria tenella* [12]. Taken together, the present findings indicate that acetic acid, cresol soup and benzene+xylene are good candidates for suppression of *E. tenella* oocyst sporulation.

**CONFLICT OF INTEREST**

The author has no conflict of interest related to this work.

**REFERENCES**

1. Daugschies A, Bose R, Marx J, Teich K, Friedhoff KT. Development and application of a standardized assay for chemical disinfection of coccidia oocysts. Vet Parasitol 2002; 103: 299-308.
2. McDonnell G, Russell AD. Antiseptics and disinfectants: activity, action, and resistance. Clin Microbiol Rev 1999; 12: 147-179.
3. Williams RB. Laboratory tests of phenolic disinfectants as oocysticides against the chicken coccidium *Eimeria tenella*. Veterinary Record 1997; 141: 447-448.
4. Rehman TU, Khan MN, Sajid MS, Abbas RZ, Arshad M, Iqbal Z. Epidemiology of *Eimeria* and associated risk factors in cattle of district Toba Tek Singh, Pakistan. Parasitol Res 2011; 108: 1171-1177.
5. Williams RB. Tracing the emergence of drug-resistance in coccidia (*Eimeria* spp.) of commercial broiler flocks medicated with decoquinate for the first time in the United Kingdom. Vet Parasitol 2006; 135: 1-14.
6. Haug A, Thebo P, Mattsson JG. A simplified protocol for molecular identification of Eimeria species in field samples. Vet Parasitol 2007; 146: 35-45.
7. Guimaraes JS, Bogado ALG, Da Cunha TCB, Garcia JL. In vitro evaluation of the disinfection efficacy on *Eimeria tenella*, unsporulated oocysts isolated from broilers. Rev Bras Parasitol Vet 2007; 16: 67-71.
8. Hilbrich P. Disinfection tests on *Eimeria tenella* oocytes. Berl Munch Tierarztl Wochenschr 1975; 88: 144-148.
9. Daugschies A, Agneessens J, Goossens L, Mengel H, Veys P. The effect of a metaphylactic treatment with diclazuril (Vecoxan®) on the oocyst excretion and growth performance of calves exposed to a natural *Eimeria* infection. Veterinary Parasitology 2007; 149: 199-206.
10. Ayeni AO, Dingeldein E, Durr U. Studies on the inactivation of coccidian oocysts. Acta Vet Acad Sci Hung 1972; 22: 111-122.
11. Grier N. Synthesis and antimicrobial evaluation of quaternary salts of 4-phenyl-1,2,3,6-tetrahydropyridine and 3,6-dimethyl-6-phenyl-tetrahydro-2H-1,3-oxazine. J Pharm Sci 1979; 68: 407-411.
12. Zaman MA, Iqbal Z, Abbas RZ, Khan MN. Anticoccidial activity of herbal complex in broiler chickens challenged with *Eimeria tenella*. Parasitology 2012; 139: 237-243.