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Research Note

Efficacy of Oral Metronidazole and Potassium Sorbate Against Two Gregarine Parasites, *Protomagalhaensia granulosae* and *Gregarina cubensis* (Apicomplexa: Eugregarinida), Infecting the Death's Head Cockroach, *Blaberus discoidalis*

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ABSTRACT: Gregarines are parasites common in insects, especially in culture. No effective chemotherapeutic or chemoprophylactic control protocol exists, but sulfadimethoxine has targeted effects on sporozoites and trophozoites. Efficacies of metronidazole and potassium sorbate were independently administered in 5-d regimens to evaluate their efficacy as chemotherapeutic agents against 2 gregarine species, *Gregarina cubensis* and *Protomagalhaensia granulosae*, infecting Death's Head Cockroaches, *Blaberus discoidalis*. Relative to the control, metronidazole significantly reduced intensities of both *G. cubensis* and *P. granulosae* but did not significantly reduce gametocyst production. Potassium sorbate had no effect on gregarine intensities but significantly increased the rate of gametocyst production. These results eliminate potassium sorbate as a chemotherapeutic agent in this system but suggest that metronidazole has promise as a gregarine control agent in arthropod monoculture.

KEY WORDS: Gregarine, chemotherapy, Apicomplexa, *Protomagalhaensia granulosae*, *Gregarina cubensis*, *Blaberus discoidalis*.

Gregarines (Protista: Apicomplexa) are the most ubiquitous and taxonomically diverse of the apicomplexan parasites, infecting a wide range of nonvertebrate hosts, but are most commonly found in arthropods, especially insects (Levine, 1988; Clopton, 2002). Gregarines have been implicated as pathological agents whose effects range from reduced host longevity (Jackson, 1985; De Montaigne and Maouloud, 1986; Zuk, 1987a, b; Lipa and Triggiani, 1989; Iperti, 1999) and reduced host fecundity (Kulka and Corey, 1984; Jackson, 1985; Zuk, 1987a, b; Thomas et al., 1999) to epidemic mortality (Purrini and Keil, 1989; Pushkala and Muralirangan 1997, 1998; Johny et al., 2000). Sanitary insectary protocols have been suggested, but satisfactory gregarine control by these methods is laborious and unpredictable (Jackson, 1985; Dougherty et al., 1991).

Mourya et al. (1985) conducted the seminal efficacy assays of sulfadimethoxine and metronidazole against *Ascogregarina culicis* (Eugregarinida: Lecudinidae) infecting larval *Aedes aegypti* (Diptera: Culicidae). Larvae were exposed environmentally, but metronidazole elicited no significant control effect. However, high concentrations of sulfadimethoxine not only reduced mean intensity in adult mosquitoes but also induced heavy host mortality. Clopton and Smith (2002) reported effective control of gregarine sporozoites and trophozoites with a 5-d oral regimen of sulfadimethoxine with no host mortality, but sulfadimethoxine had no significant effect on gamont prevalence or gametocyst production in their gregarine–cockroach model system.

This study evaluates the chemotherapeutic efficacy of metronidazole and potassium sorbate against 2 gregarine species, *Gregarina cubensis* and *Protomagalhaensia granulosae,* infecting the Death's Head Cockroach *Blaberus discoidalis*. Metronidazole, 1-(beta-hydroxyethyl)-2-methyl-5-nitroimidazole, is an imidazole derivative belonging to a class of antimicrobial agents widely used for chemotherapeutic treatment of human infection with a variety of protists and anaerobic bacteria. Potassium sorbate is a potassium salt of sorbic acid commonly used as a food preservative. When in contact with water, potassium sorbate ionizes to form sorbic acid, a fungistatic and bacteriostatic agent of variable efficacy against yeasts, molds, bacteria, and some protists.

A colony of *B. discoidalis* was established using stock purchased from Carolina Biological supply (Burlington, North Carolina, U.S.A.). Initial colony survey indicated 100% prevalence of *P. granulosae* and *G. cubensis*. For each of the 3 replications, 3 treatment groups of 7 cockroaches each were maintained in individual plastic shoeboxes with wood chip bedding. All groups were supplied with dog food and a water source. The control group was administered a normal insect saline solution (Belton and Grundfest, 1962). The metronidazole test group was administered an oral suspension of metronidazole (0.4 mg/kg

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Table 1. Mean intensities (± SD) of *Protomagalhaensia granulosae, Gregarina cubensis,* and gregarine gametocysts from *Blaberus discoidalis* under 3 treatment regimens.*

Treatment	*P. granulosae*	*G. cubensis*	Gametocysts
Control†	183.76 ± 54.49	162.76 ± 47.55	0.38 ± 1.12
Metronidazole	53.14 ± 30.44‡	41.43 ± 29.18‡	0.24 ± 0.54
Potassium sorbate	187.24 ± 92.7	150.48 ± 73.98	5.90 ± 13.77‡

* Overall mean of 3 replications per treatment; 7 cockroaches per replicate; treatment $N = 21$).

† Control group administered saline.

‡ Significantly different from other treatments in the column (ANOVA protected Tukey's honestly significant difference test, $\alpha = 0.05$).

bodyweight), and the potassium sorbate test group was administered an oral solution of potassium sorbate (0.0152 mg/kg bodyweight). Metronidazole and potassium sorbate drug dosages reflect existing recommendations for treatment of commercial livestock and preservation of baked and dairy goods, respectively. Treatments were administered each day for 5 d as follows: individual cockroaches were held upside down and dosed by placing drops of solution on the cockroach's mandibles with a 26-gauge needle and 5-cc syringe. Cockroaches were allowed to completely ingest each drop before subsequent drops were administered. The process was repeated drop-wise until individuals consumed the daily treatment dose. On day 6, all cockroaches were killed and examined for gregarine infection. Individuals of *P. granulosae* and *G. cubensis* were identified using the comparative morphological descriptions of Peregrine (1970) and were tabulated. Gametocysts of both species recovered from the posterior midgut and rectum were counted and incubated for development and dehiscence as described by Clopton (1996).

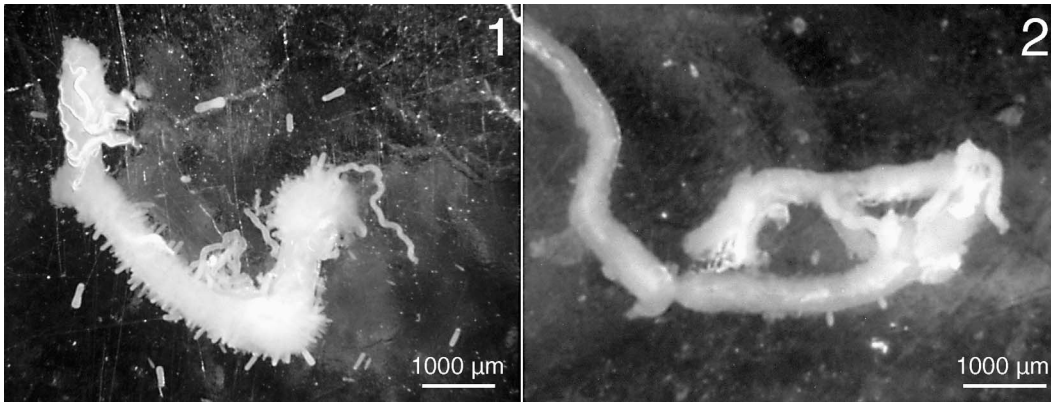
Data were analyzed using univariate analysis of variance, blocking on treatment and replication. Differences among treatment groups were identified using Tukey's honesty significant difference test. All analyses were completed using SPSS Base 10.0 (SAS Institute, Carey, North Carolina, U.S.A.) with $\alpha = 0.05$.

Mean intensities of *P. granulosae, G. cubensis,* and gregarine gametocysts from *B. discoidalis* control, metronidazole, and potassium sorbate treatment groups are presented in Table 1. The intensity of *P. granulosae* in the cockroaches treated with metronidazole was significantly lower than that observed in control group cockroaches or cockroaches treated with potassium sorbate. There was no significant difference in *P. granulosae* intensities observed in control group cockroaches and cockroaches treated with potassium sorbate. Likewise, intensity of *G. cubensis* within the ceca of cockroaches treated with metronidazole was significantly lower than that observed in control group cockroaches or cockroaches treated with potassium sorbate. Figures 1 and 2 depict typical ceca from a control group cockroach and a cockroach treated with metronidazole, respectively, demonstrating the dramatic reduction in gregarine intensity produced by metronidazole chemotherapy. Metronidazole treatment did not significantly reduce production of gametocysts but treatment with potassium sorbate significantly increased gametocyst production. Gametocysts from all treatment groups underwent normal development and dehiscence.

Gregarine mean intensities (Table 1) demonstrate a reduction in the numbers of *P. granulosae* and *G. cubensis* in cockroaches receiving metronidazole chemotherapy. Metronidazole reduced gregarine mean intensities by 71%, an effect comparable with the 80% reduction reported by Clopton and Smith (2002) for treatment with sulfadimethoxine in the same gregarine–cockroach experimental model. The continued production of gametocysts by cockroaches in the metronidazole treatment group suggests that metronidazole acts against early ontogenic stages rather than against all endogenous stages of the gregarine life cycle. Such a pattern of ontogenic specificity reduces overall gregarine intensity by halting sporozoite and trophozoite development. Lack of a significant effect on gametocyst production reflects the normal completion of the life cycle by existing gregarine populations that had already passed through the developmental window in which metronidazole acts. This pattern of effect is identical to that reported for sulfadimethoxine by Clopton and Smith (2002).

In contrast to metronidazole, potassium sorbate had no effect on gregarine mean intensities (Table 1) and is not a candidate for control of gregarine infection in insect colonies. The significant increase in gametocyst production observed with potassium sorbate treatment is enigmatic. We are at a loss to posit a likely mode of action for this effect.

This study demonstrates the efficacy of metronidazole as a gregarinostat. Metronidazole appears to abort the development of gregarine sporozoites, trophozoites, and early-stage gamonts without disrupting the ability of late-stage gamonts to form gametocysts. Gametocysts formed in hosts treated with metronidazole appear to develop normally and produce normal oocysts, but the viability of these oocysts was not tested, and thus the effect of metronidazole on oocyst viability remains unknown. In a manner similar to that suggested for sulfadimethoxine by Clopton and



Figures 1–2. Reduction in *Gregarina cubensis* intensity produced by metronidazole treatment of the Death's Head Cockroach, *Blaberus discoidalis*. **1.** Single intestinal cecum from a control group cockroach dissected to demonstrate gregarine intensity. Gregarine trophozoites attached to the cecal epithelium lend a papillated appearance to the inner surface of the cecum. **2.** Single intestinal cecum from a cockroach treated with metronidazole dissected to demonstrate gregarine intensity. Although a few gregarine trophozoites are present, the inner surface of the cecum presents its more typical smooth appearance.

Smith (2002), the stage-specific gregarinostatic action of metronidazole suggests that prophylactic control of gregarine infection in arthropod monoculture is possible if treatment is administered for a period extending beyond the life expectancy of a single gregarine cohort. Although gregarine-free insect culture will require the combination of chemotherapy and strict sanitary protocols to eliminate oocysts, periodic use of metronidazole should substantially reduce gregarine mean intensity in insect cultures.

Guhl (1999) suggested that cockroaches might be used as experimental models to test products designed to eliminate ectoparasites. We suggest that a gregarine–cockroach model system such as the one presented in this study may have greater utility as a tool to screen the pharmacological action of potential chemotherapeutic agents. This is particularly important in the case of parasites such as species of *Cryptosporidium* that have significant human impact but for which there exists no inexpensive and efficacious screening system. Based on small-subunit ribosomal ribonucleic acid gene sequences, Carreno et al. (1999) concluded that gregarines and *Cryptosporidium* are sister groups, forming a clade that is distinct from other apicomplexan clades. This close relationship between gregarines and *Cryptosporidium* suggests that gregarines hold some promise as model systems for *Cryptosporidium*. Insect–egarine models are inexpensive relative to mammalian or cell culture screening models and might be used as target organisms for screening pharmacologically active compounds for use against *Cryptosporidium*.

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Research Note

Helminths and Ectoparasites of *Rattus rattus* and *Mus musculus* from Sicily, Italy

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ABSTRACT: We investigated the endo- and ectoparasitic fauna of 45 specimens of *Rattus rattus* and 44 specimens of *Mus musculus* collected in Sicily. This study is the second survey of the parasitic fauna of these 2 rodent species on this Mediterranean island. Four nematode species, 1 cestode species, 2 flea species, and 1 tick species were recovered from *R. rattus*. Six nematode species, 3 cestode species, 1 digenean species, 1 flea species, and 1 tick species were

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