Abstract

Pythium insidiosum, an aquatic oomycete*,* with a biflagellate infective zoospore stage that causes invasive, progressive, life-threatening granulomatous dermatological and gastrointestinal disease in dogs, cats and horses. Historically, pythiosis has been thought of a disease of tropical and subtropical climates. However, in the past two decades, its environmental niche has expanded to include more temperate (north) and more arid (southwest) regions of the United States of America. This phenomenon is potentially associated with an expanding geographical distribution and to the development of advanced diagnostics such as isolation of *P. insidiosum* by culture and/or by serological and PCR techniques.

Clinical signs are related to zoospores that encyst in either damaged skin or the gastrointestinal mucosa leading ulcerative nodular lesions with draining tracts and vomiting, progressive weight loss, anorexia, and diarrhea respectively. Infection is characterized by rapid clinical progression with a high mortality rate due to extensive, but typically localized, spread of the disease.

Treatment is challenging and there is no consensus on standard treatment recommendations. Standard treatment recommendations include complete surgical resection of affected tissues with long-term conventional adjunctive antifungal therapy, though local recurrence is commonly reported. Variable and unpredictable response to this adjunctive therapy has seen the need to explore the use of other compounds as alternative options for the treatment of *P. insidiosum* infections in animals.

Malachite green, an *N*-methylated diaminotriphenylmethane dye, has been traditionally used as an antifungal agent in commercial fish hatcheries and in the control of oomycetes in plants. Malachite green works as a photosensitizer, absorbing light and initiating the formation of free radicals, resulting in inhibition in the growth of *P. insidiosum*. Additional proposed mechanisms of action include inhibition of intracellular enzymes, intercalation into DNA, and/or intercalation into cell membranes.

Recently we have performed an in vitro study showing the potential effect of Zn-associated polyammonium bisulfate molecular clusters (PBMC) and nanosulfur against clinical isolates of canine *P. insidiosum*. Thus, the goal of this study is to test a higher concentration of Zn-PBMC in association with nanosulfur against canine *P. insidiosum* as preliminary study to use the best combination of PBMC/nanosulfur for a clinical trial on cutaneous canine pythiosis.

Specific aim

The primary aim of this study is to investigate the *in vitro* activity of Zn- and/or Cu-associated polyammonium bisulfate molecular clusters (PBMC) and nanosulfur against canine isolates of *Pythium insidiosum* previously isolated from canine patients at the University of Florida Small Animal Hospital.

Student role

The selected student will be directly involved in the actuation of the project. The student will perform the microbiological assays. Once collected, data will be analyzed and the results evaluated. The student will be involved in scientific writing and publication.