

ALMA MATER STUDIORUM Università di Bologna Dipartimento di scienze mediche veterinarie

DOTTORATO DI RICERCA in Scienze Veterinarie XXXVI CICLO - A.A. 2021/2022 CURRICULUM: Produzioni Animali e Sicurezza Alimentare DOTT. SSA Martina Felici TUTOR: PROF. Andrea Piva



Understanding the anticoccidial mode of action of a thymol-based botanical blend

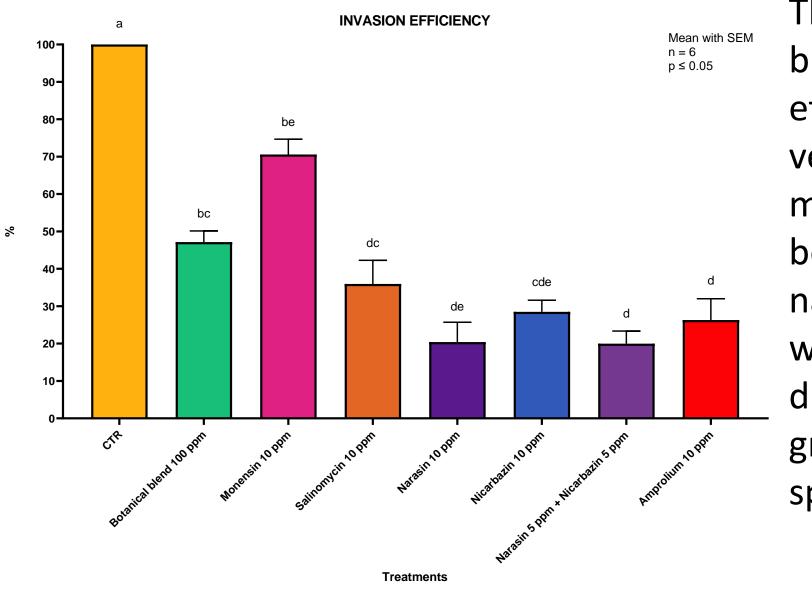
Background

Objectives

Coccidiosis is a major problem in poultry production, leading to significant economic losses. Due to the outbreak of resistance to the available treatments, research is focusing on finding new molecules that work against the pathogen. Botanical compounds represent promising alternatives, but reliable *in vitro* tests are needed for their screening and to explore their mechanism of action.

Results

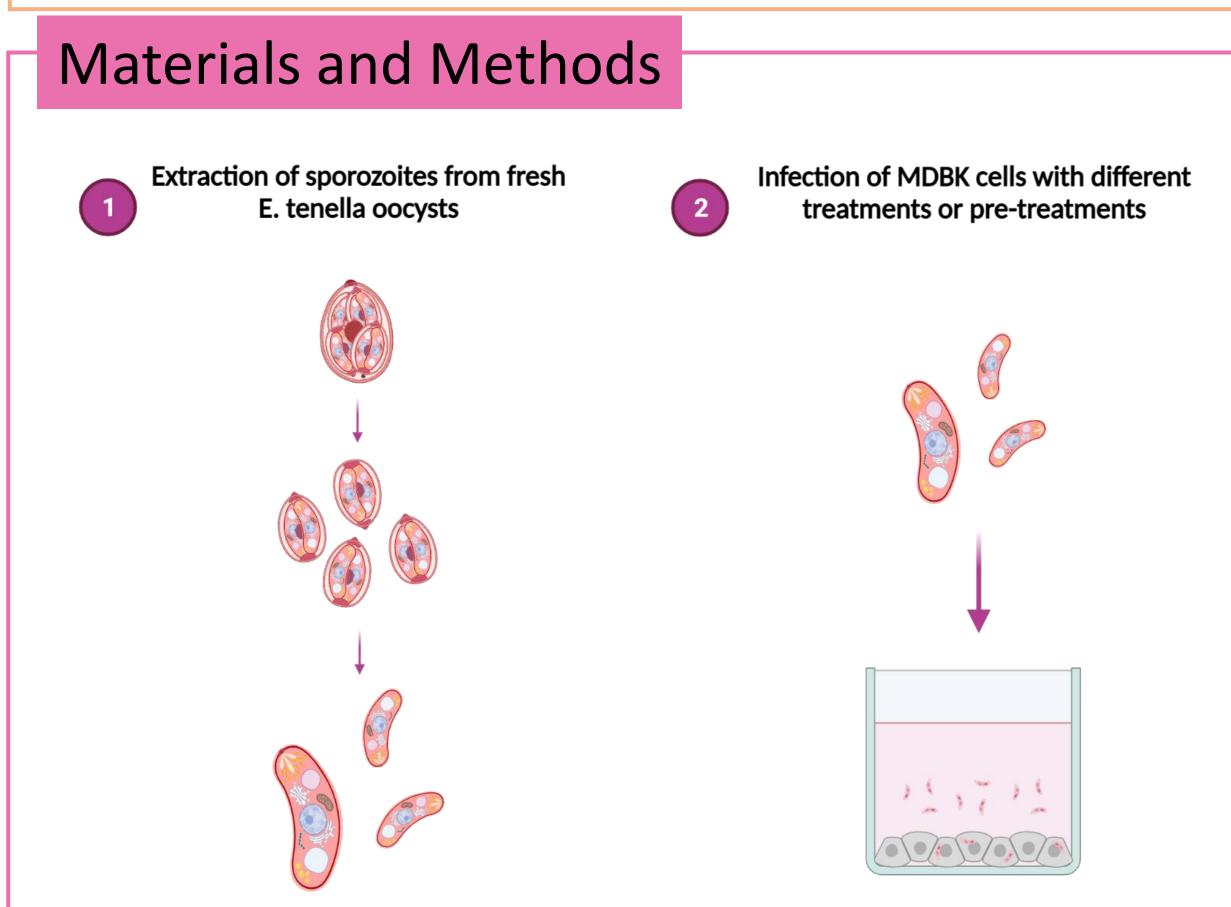
Test #1: incubation for 24 h with different anticoccidials and a botanical blend to **compare the efficiencies of invasion on MDBK cells**



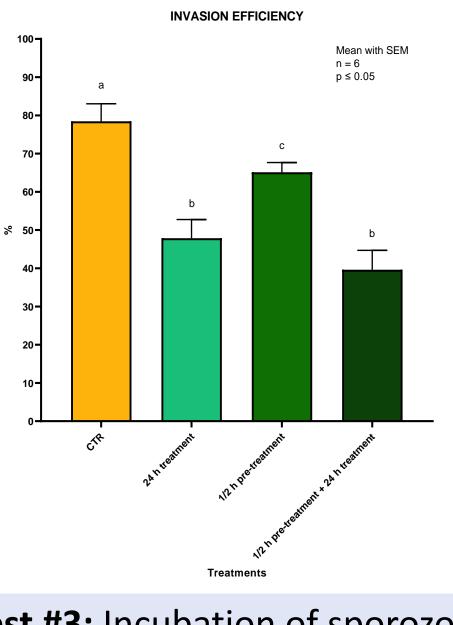
The thymol-based botanical inhibits blend invasion efficiency by 50% and it is very similar to salinomycin; it moreover, stands in between monensin and ionophores, all narasin, which anticoccidial are drugs that disrupt the ion gradient leading to sporozoite death.

The objective of this study is to evaluate the anticoccidial efficacy and mode of action of a thymol-based botanical blend, in order to:

- 1. Compare the efficacy to anticoccidial drugs
- 2. Understand how fast the anticoccidial action is completed
- 3. Understand the mode of action of the botanical blend by morphological observations



Test #2: pre-treatment for ½ h and invasion assay on MDBK cells to understand the **kinetics of action of the botanical blend**



In this test, the anticoccidial efficacy of the thymol-based botanical blend after 24 h of treatment was confirmed. The pre-treatment with the same blend also significantly inhibited invasion of *E. tenella* sporozoites by 17%.

In addition, the invasion efficiency of the pre-treatment, followed by the 24h treatment was investigated, and a tendency to further decrease the efficiency of invasion was found.

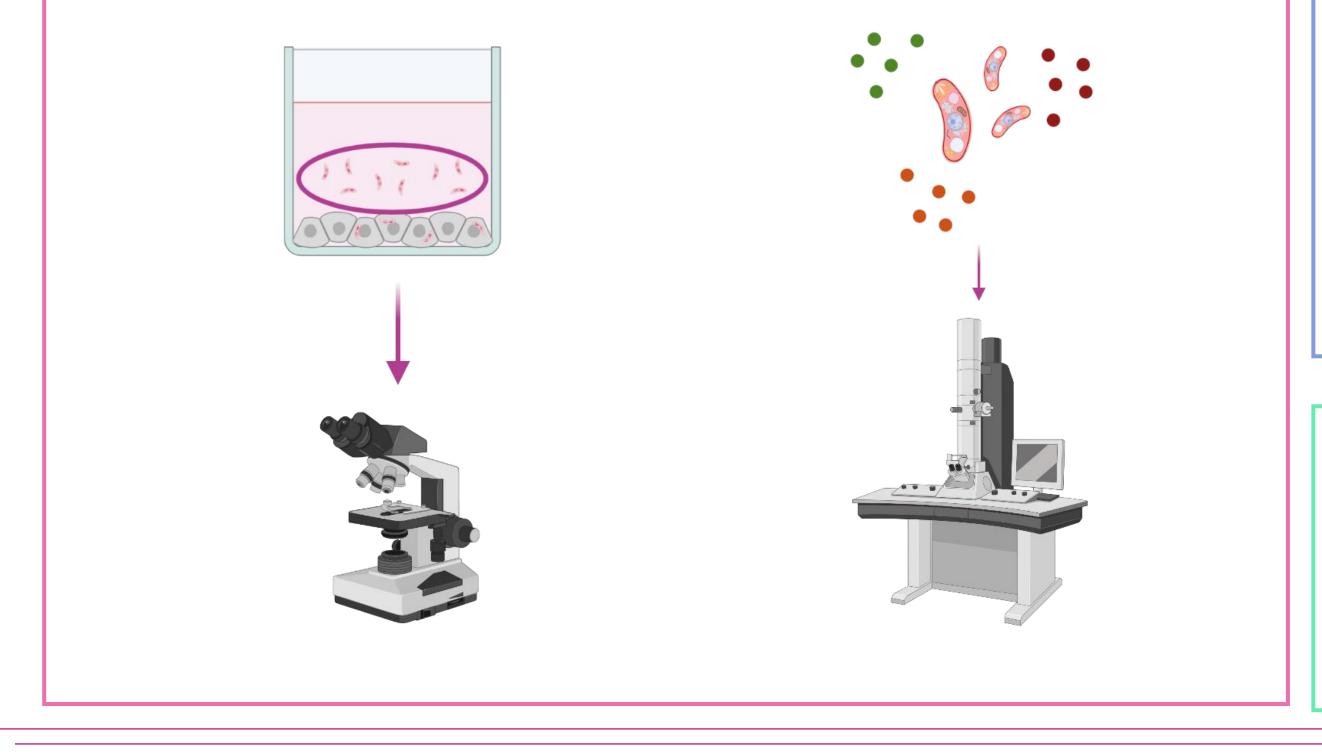
Test #3: Incubation of sporozoites in the botanical blend and salinomycin for 2 h and **detection of morphological changes by scansion elecron**

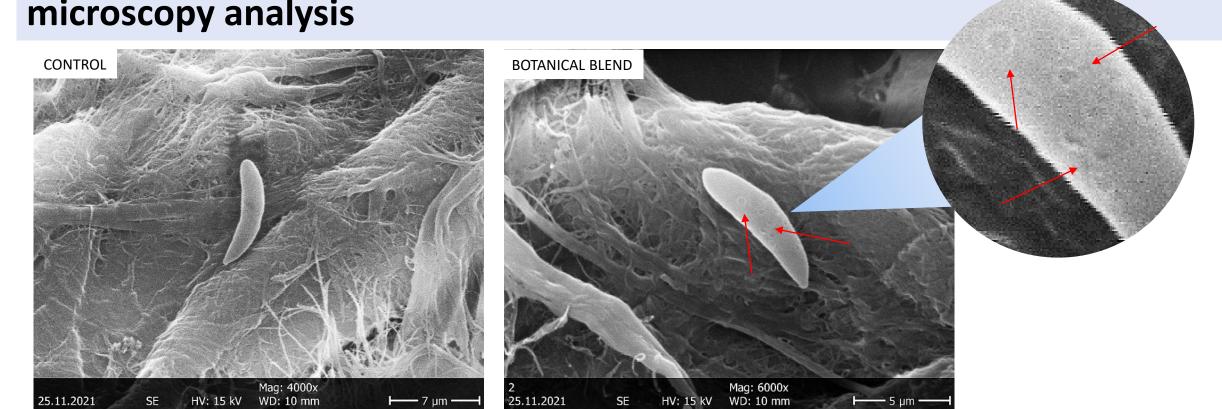


Counts of extracellular sporozoites to determine invasion efficiencies



Incubation of different treatments with sporozoites for scansion electron microscopy analysis





Unlikely the untreated group, the cytoplasmatic membrane of treated sporozoites showed the presence of holes, in both treatments.

Conclusions

These findings suggest that the studied botanical blend has a rapid anticoccidial efficacy, and it significantly inhibits the invasion of *E. tenella* already after ½ h of pre-treatment. The specific mode of action is linked to the creation of holes on the sporozoites' membranes, similarly to ionophore anticoccidials.

Papers

Felici M, Tugnoli B, Piva A, Grilli E. In Vitro Assessment of Anticoccidials: Methods and Molecules. Animals (Basel).

2021;11(7):1962. Published 2021 Jun 30. doi:10.3390/ani11	J/1962
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