



## BACKGROUND

- *Clostridioides difficile* infection (CDI) is an urgent public health threat worldwide and a significant financial healthcare burden (1)
- Secondary bile acids (SBA) play an important role in maintaining mucosal integrity, promoting wound healing, and their absence is associated with dysbiosis and germination and/or growth of *C. difficile* (2)
- Previous studies have shown that ursodeoxycholic acid (UDCA), a commercially available secondary bile acid, inhibits germination and growth of *C. difficile* in vitro (3)
- Based on known CDI pathogenesis and results from previous studies, it can be hypothesized that UDCA could have a role in protecting against CDI
- Observational studies evaluating the use of UDCA in humans and *in vivo* animal experiments have shown conflicting effects (2,3)
- The invertebrate model *Galleria mellonella* has become an attractive alternative to other *in vivo* models in infectious diseases-related research, including bacterial and fungal virulence, viral infections, and antimicrobial screening and testing (4)
- This popularity is attributed to its low cost, short life cycle, simple handling, and lack of ethical constraints (4)
- The effect of UDCA has not been investigated in a simple *in vivo* model such as *G. mellonella*.

## OBJECTIVE

- To evaluate the effects of UDCA on *C. difficile* germination and growth *in vitro* and on survival using a *G. mellonella* model

## METHODS

### Growth and germination of *C. difficile*:

- *C. difficile* spores were incubated in anaerobic chamber with Brain heart infusion (BHI) alone and with different concentrations of UDCA (0.001, 0.01, 0.05, and 0.1%)
- After 24 hours of incubation, optical density (OD) was measured using BHIS + 2% ethanol as a control

### Larval inoculation and treatment:

- Larvae were gavaged (force fed) with  $1 \times 10^5$  CFU of one *C. difficile* ribotype (RT) 027 strain (CD 196) and one RT 014-020 strain (MT-5313)
- Larvae were pretreated with different doses of UDCA via gavage 30 minutes prior to *C. difficile* inoculation
- The larvae were kept at 37°C post-infection and monitored daily for 120 hours for survival
- Larvae were assigned into the following arms and experiments were repeated in duplicate:
  1. Negative control (PBS only), n = 40
  2. Positive control (*C. difficile* only; n = 20 for each strain), n = 40
  3. UDCA (12,500 ug) + *C. difficile* inoculation (n = 20 for each strain), n = 40
  4. UDCA (12.5 ug) + *C. difficile* inoculation (n = 20 for each strain), n = 40
  5. UDCA (0.0125 ug) + *C. difficile* inoculation (n = 20 for each strain), n = 40

Figure 1. Gavaging of *G. mellonella*



## RESULTS

Figure 2. Optical densities (OD) after 24 hours of *C. difficile* growth

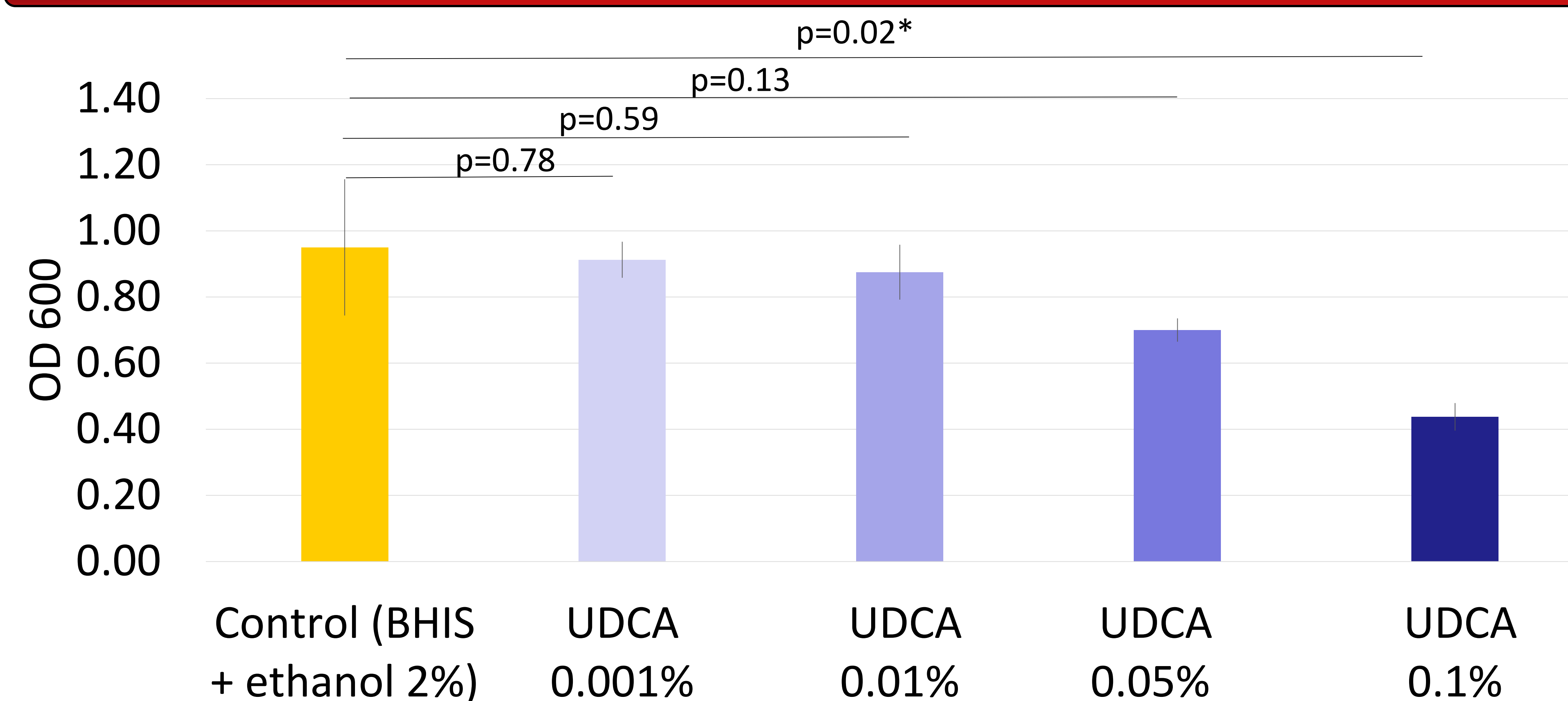
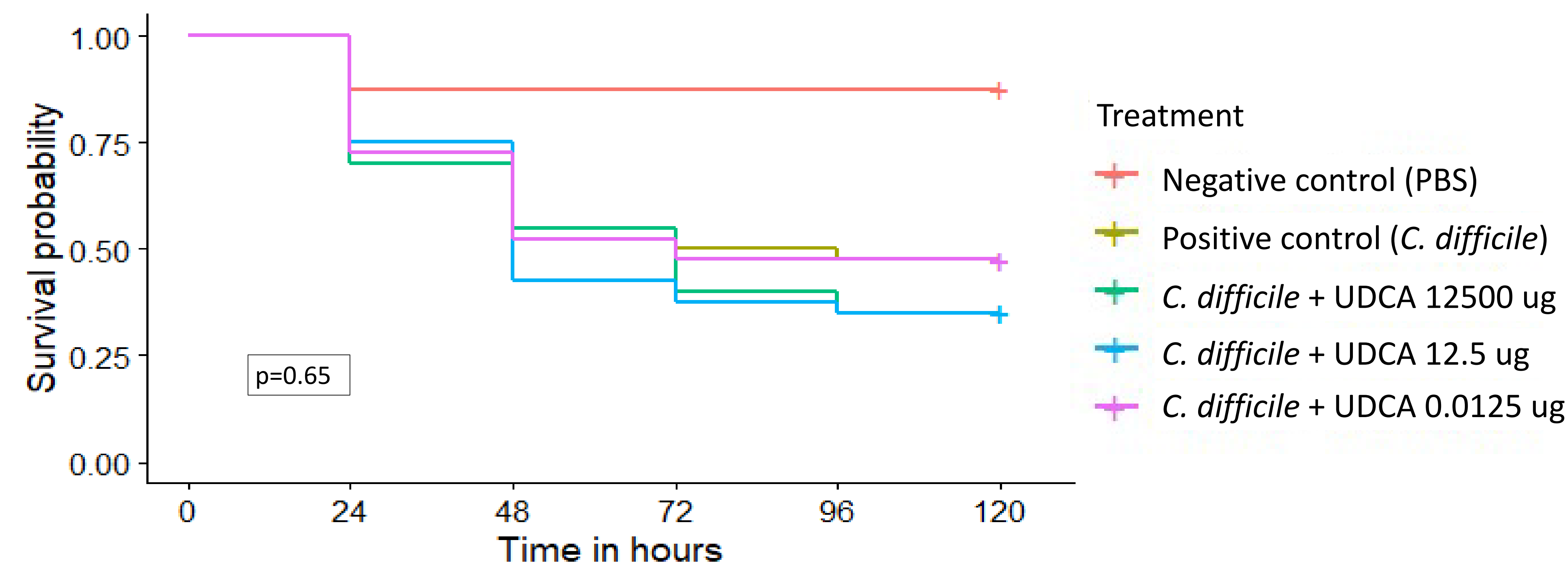


Figure 3. Larvae overall survival (n=200)



## CONCLUSIONS

- UDCA demonstrated an inhibitory effect on *C. difficile* growth and germination *in vitro*
- In our *G. mellonella* model, UDCA did not show a significant difference in preventing CDI
- Further investigation to explore the role of UDCA in CDI is warranted

## REFERENCES

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