

BACKGROUND

- Arsenic is a group one human carcinogen and potent cytotoxin.¹ Globally, over 100 million people are exposed to unsafe inorganic arsenic (iAs) levels, primarily via contaminated drinking water.¹ In human exposures, there is a high degree of interindividual variability among similarly exposed individuals.²
- Previous studies from the Walk Lab demonstrated the importance of the gut microbiome in protecting against arsenic toxicity in murine models.³ Based on this, the Walk Lab has established a murine model co-exposed to inorganic arsenic and the broad spectrum antibiotic Cefoperazone (Cef) that recapitulates the interindividual susceptibility observed in humans.⁴ To our knowledge, this is the first whole-organism model to do so.
- Currently, it is not clear what drives this interindividual difference between mice.

OBJECTIVE

- Here, we further explored differences between sick and healthy mice co-exposed to iAs and Cefoperazone to better understand what drives these interindividual susceptibilities to arsenic toxicity in our model.

METHODS

Mouse Model

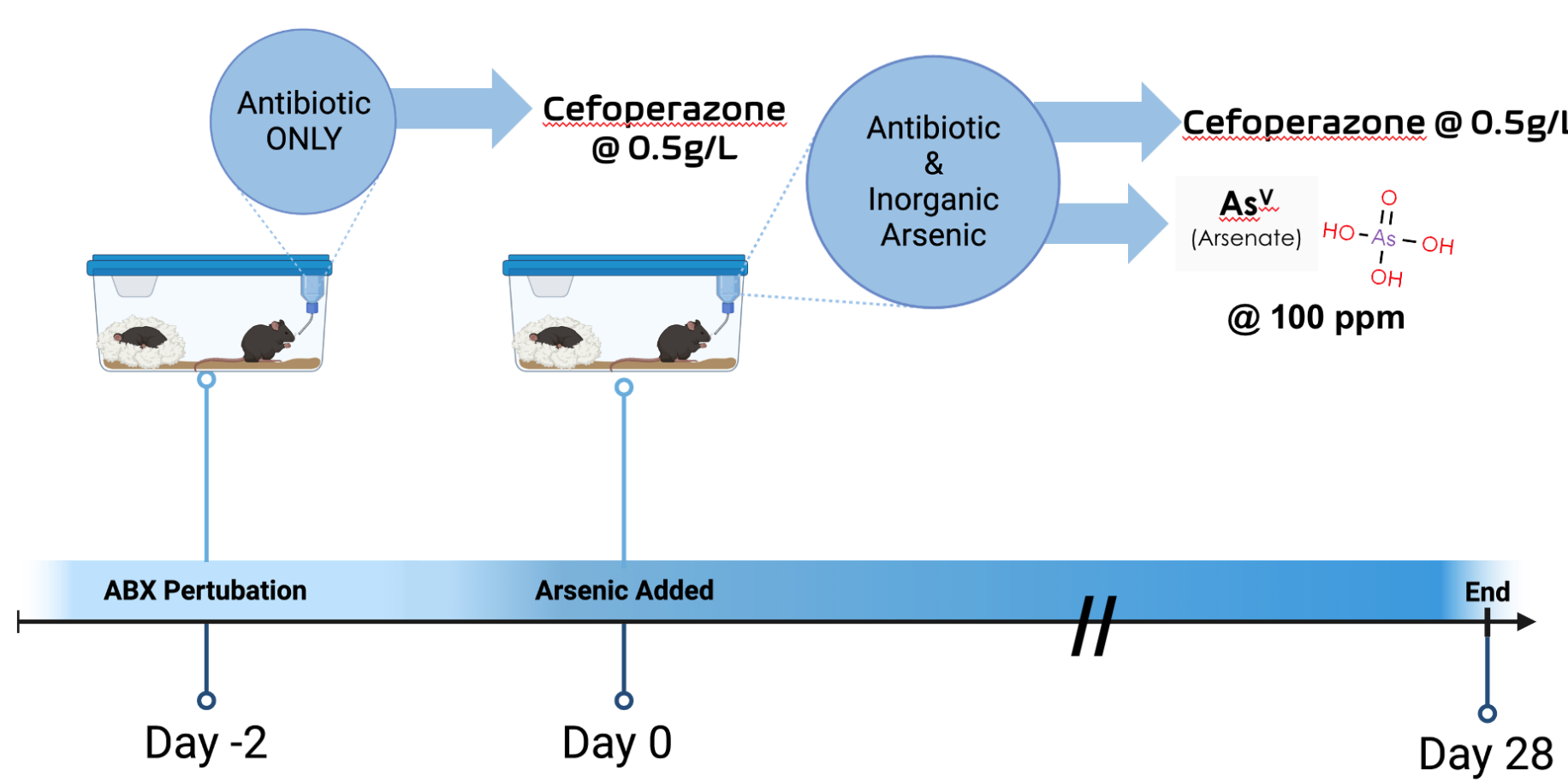


Figure 1: Mice in the Cef & iAs treatment group were treated as illustrated above. Mice in the Cef Only group were treated similarly but were not given arsenate during the experiment. Mice in the iAs Only group didn't receive Cef but were given only arsenate for 28 days. Mice in the SHAM and iAs were given heat-denatured (autoclaved) cefoperazone in addition to arsenate, starting on day 0 and ending on day 28. Daily health checks using our previously published scoring system were used to determine humane endpoints for the mice. Water supplies were exchanged for freshly made ones two times per week. Created with BioRender.com.

Blood Biochemistry, RNA Extractions, and RT-qPCR

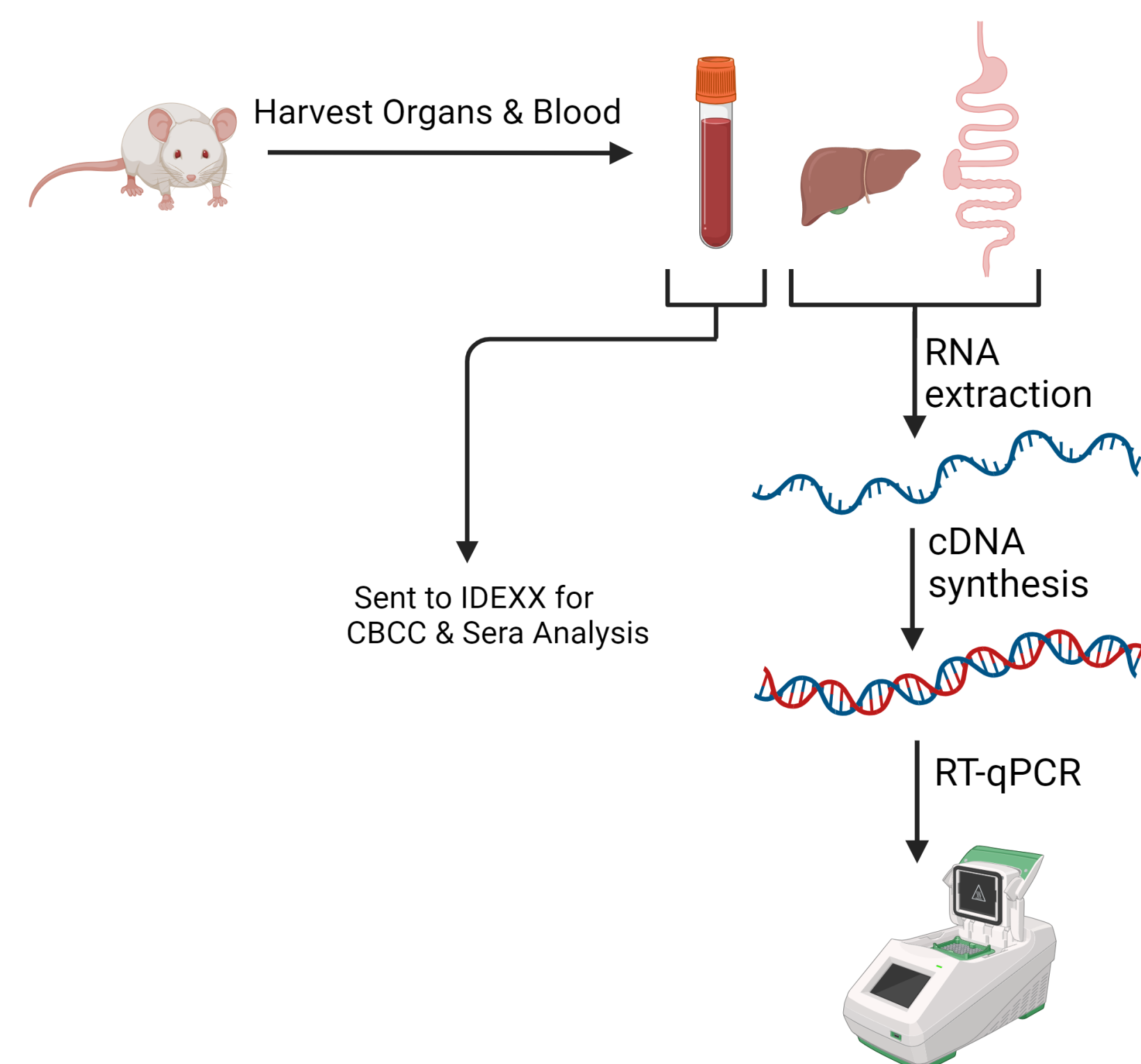


Figure 2: Blood and sera samples were collected and sent to IDEXX BioAnalytics (Sacramento, CA) for complete blood cell counts (CBC) and sera quantification for AST, ALT, and BUN. Liver and cecal samples were also collected. RNA extractions were performed, followed by cDNA synthesis and RT-qPCR to analyze gene expression levels for aquaporins (ceca) or cytokines (livers). Created with BioRender.com.

Model Outcomes

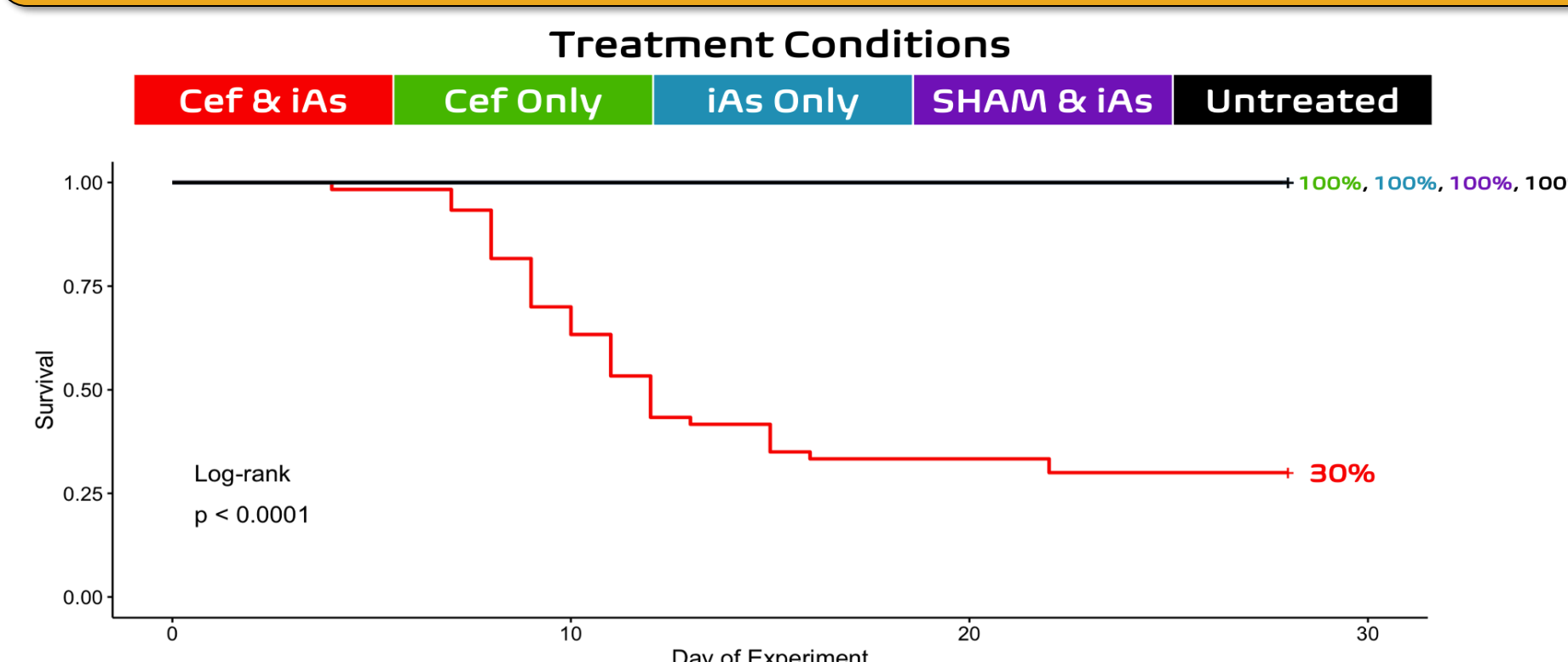


Figure 3: Kaplan-Meier survival curves for mice in each treatment group. The colors indicate what treatment group the mice were in during the experiment. Mortality was only seen at an interindividual level in the Cef & iAs treated group (30% survival). All control groups had 100% survival with no signs of disease.

Blood Biochemistry

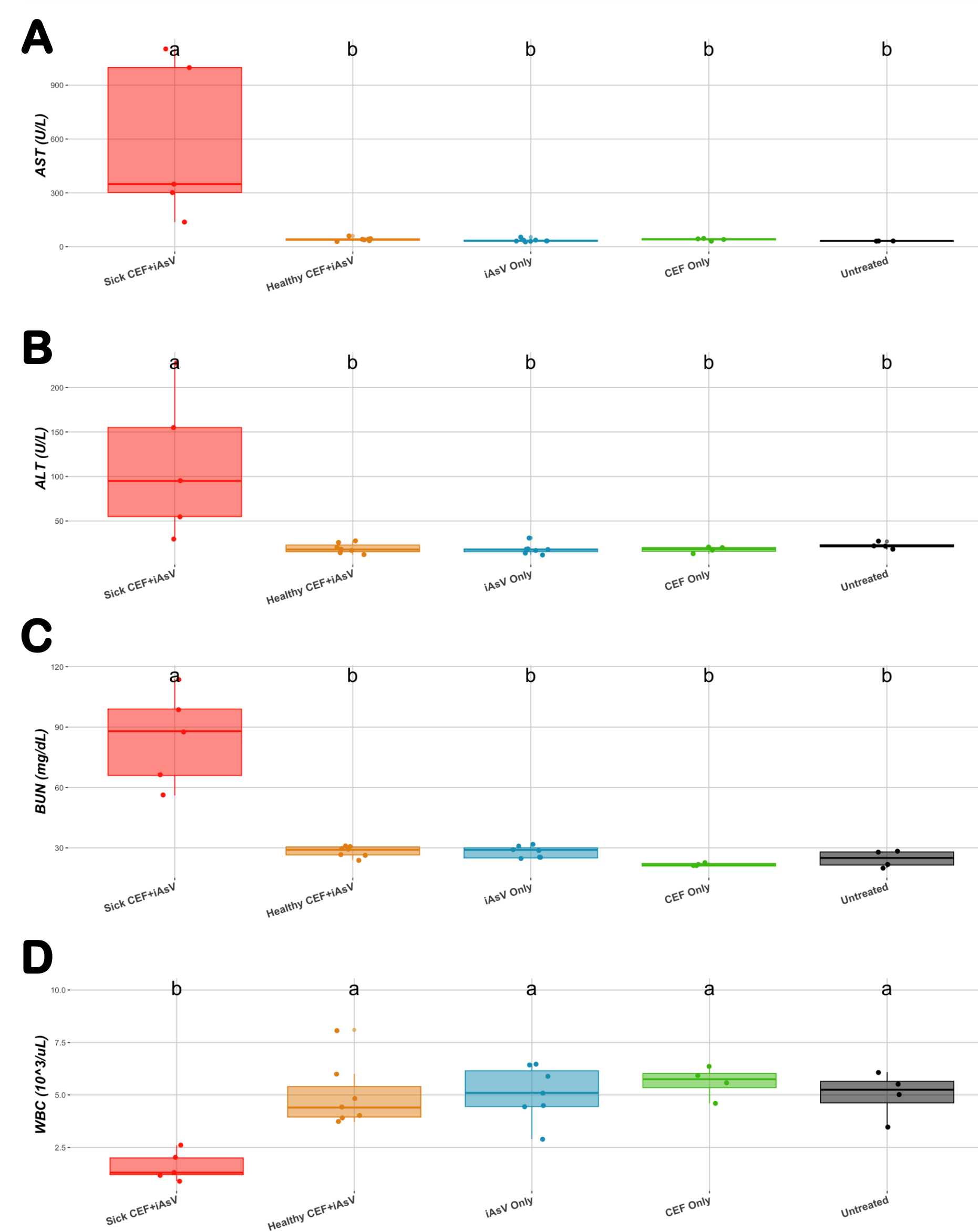


Figure 4: Blood and sera analysis. The colors indicate what treatment mice were given during the experiment. **A-C** Sera chemistry of **A.** aspartate transaminase (AST), **B.** alanine transaminase (ALT), and **C.** blood urea nitrogen (BUN). In our sera analysis, sick mice in the Cef & iAs treatment group (red) had significantly ($p < 0.05$) higher levels of AST, ALT, and BUN compared to healthy mice and all three control groups. **D.** White blood cell (WBC) counts from whole blood samples. Sick mice in the Cef & iAs treatment group (red) had significant ($p < 0.05$) low WBC counts compared to healthy mice and the three control groups. Compact letter displays at the top of each chart illustrate significant ($p < 0.05$) pairwise differences between groups per Tukey's Honestly Significant Different (HSD) method based on ANOVA. These boxplots depict the median, upper, and lower quartiles, with whiskers extending to 1.5 times the interquartile range, while individual samples are represented as dots.

RESULTS

Gross Anatomical Differences

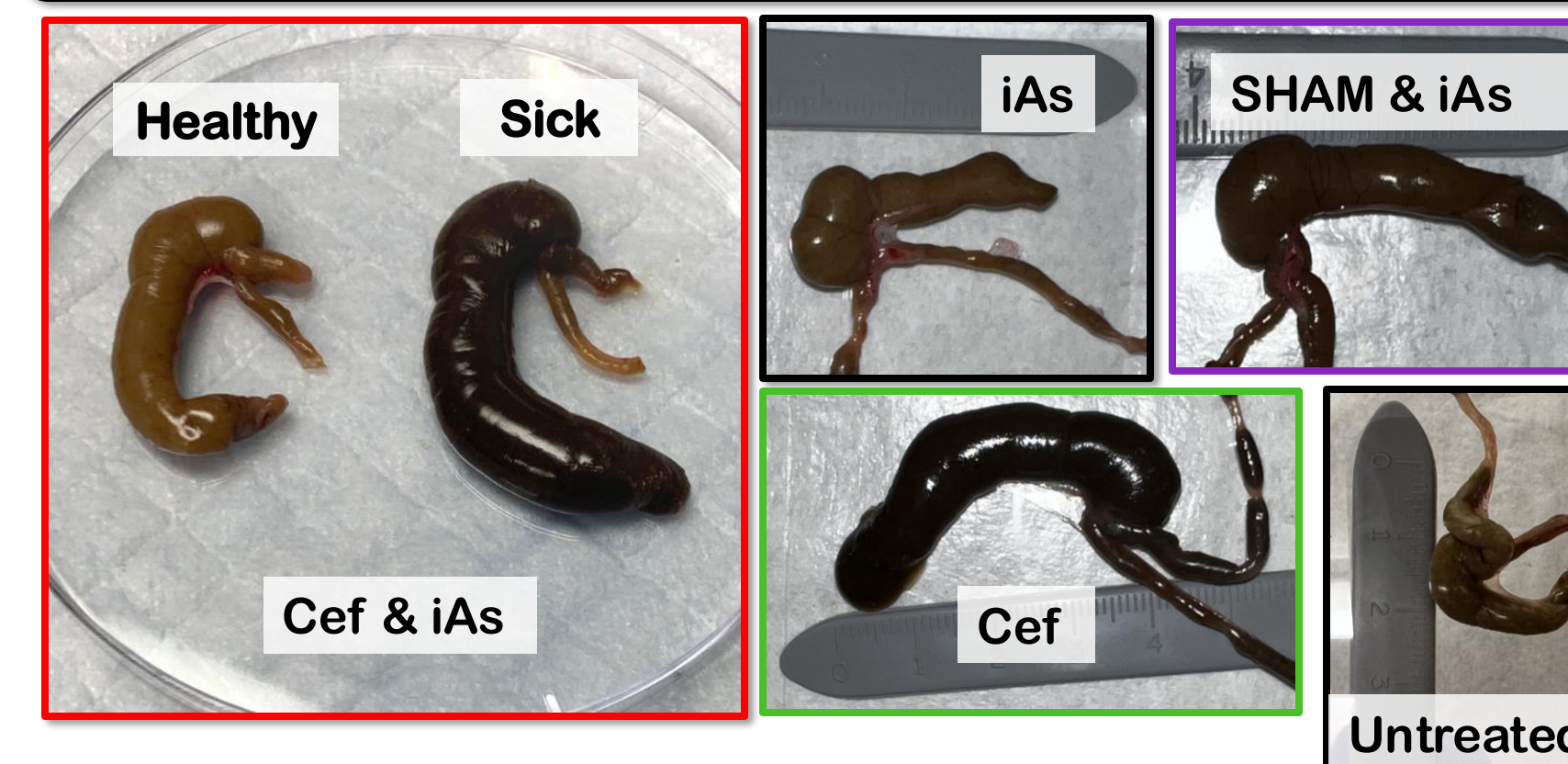


Figure 5: Gross anatomical differences of ceca. The colors indicate which treatment group the mice were in during the experiment. Ceca from healthy mice in the Cef & iAs treatment group have normal cecal anatomy similar to those from untreated mice, SHAM & iAs treated mice, and iAs-treated mice. Ceca from sick mice in the Cef & iAs treatment group are edematous, fluid-filled, and appear necrotic. Ceca from Cef-treated mice are also slightly edematous but these mice did not present with any symptoms of disease.

Expression of Aquaporins in the Cecum

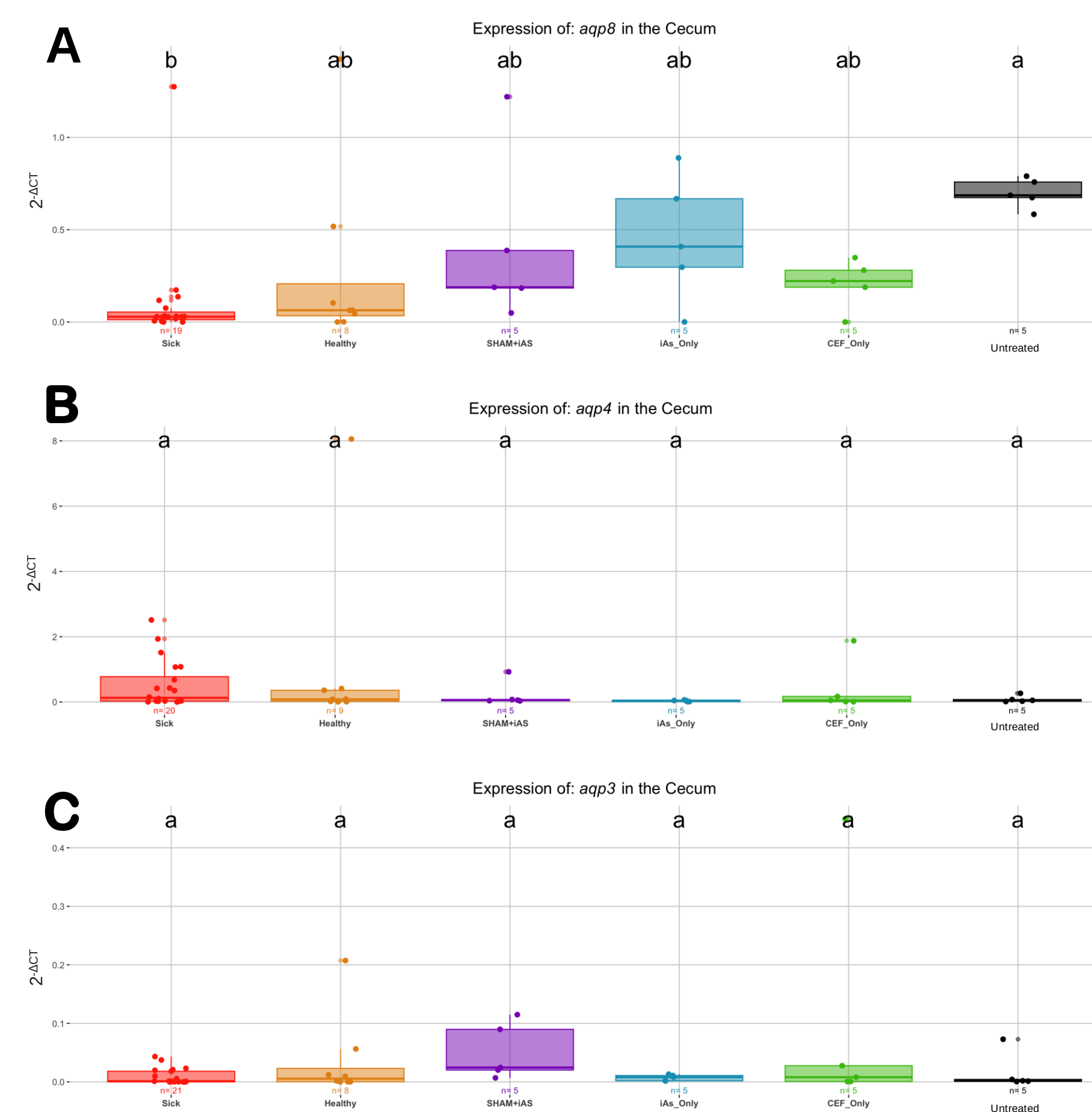


Figure 6: RT-qPCR of cecum samples assessing aquaporin expression. The colors indicate which treatment mice were given during the experiment. **A-D** RT-qPCR results of cecal samples assessing expression levels of **A.** Aquaporin 8, **B.** Aquaporin 4, **C.** Aquaporin 3. Aquaporin expression was not significantly different between sick and healthy mice in the iAs & Cef treatment group. Expression of aquaporin 8 is increased in untreated mice relative to Cef & iAs treated sick mice by a multiplicative factor of 6.5. Differences in aquaporin expression between treatment groups do not show an apparent pattern. Compact letter displays at the top of the charts illustrate significant ($p < 0.05$) pairwise differences between groups per Tukey's Honestly Significant Different (HSD) method based on ANOVA. These box plots depict the median, upper, and lower quartiles, with whiskers extending to 1.5 times the interquartile range. Dots represent individual samples.

Expression of Inflammation Markers in the Liver

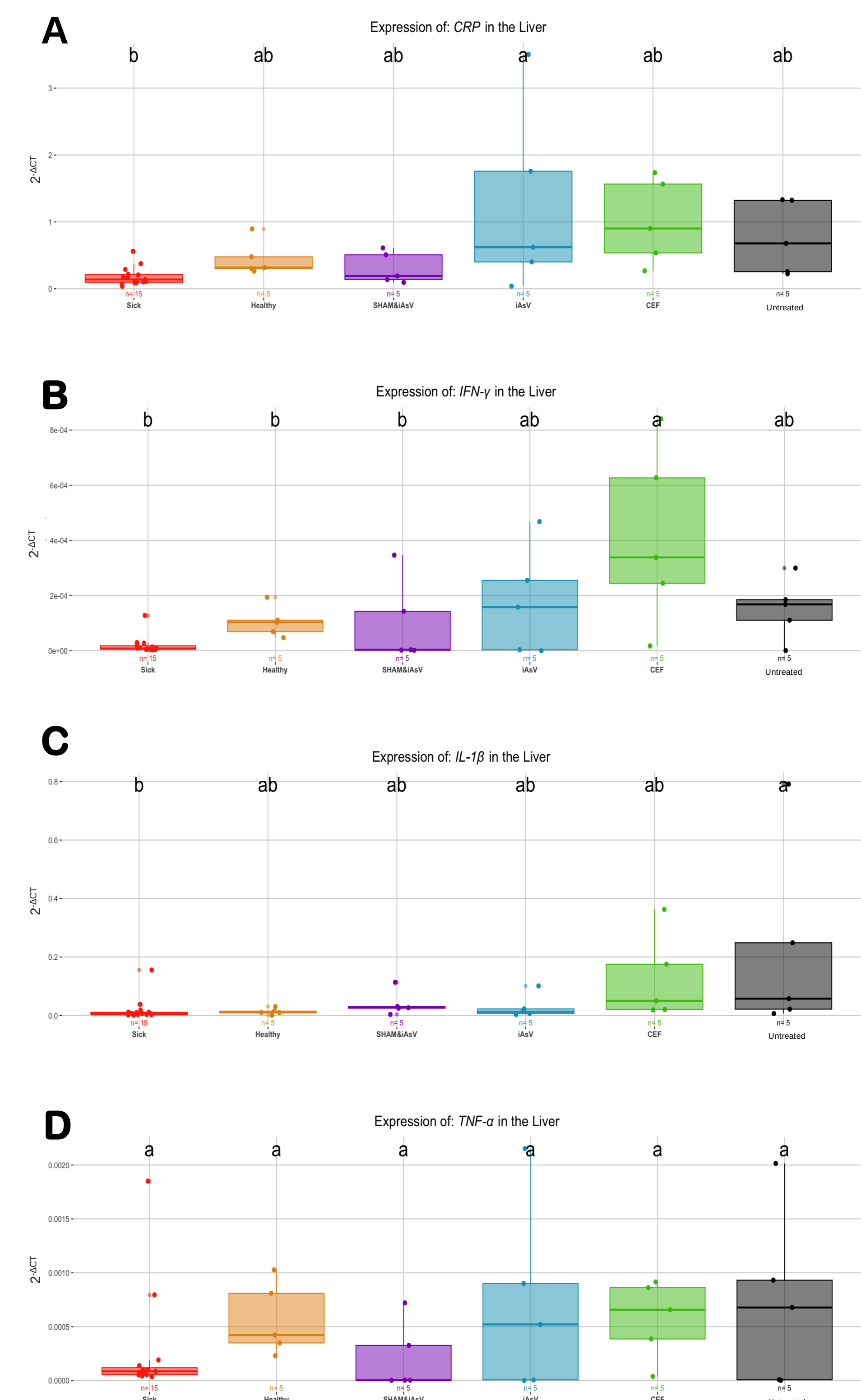


Figure 7: RT-qPCR of liver samples assessing cytokine expression. The colors indicate which treatment mice were given during the experiment. **A-D** RT-qPCR results of liver samples assessing expression levels of **A.** C-reactive Protein (CRP), **B.** Interferon gamma (IFN-γ), **C.** Interleukin-1 beta (IL-1β), **D.** Tumor necrosis factor alpha (TNF-α). Cytokine expression was not significantly different between sick and healthy mice in the Cef & iAs treatment group for any of the four cytokines. However, expression of CRP is increased in mice treated with iAs relative to Cef & iAs treated sick mice by a multiplicative factor of 6.9. Additionally, expression of IFN-γ is increased in mice treated with Cef relative to Cef & iAs treated sick mice by a multiplicative factor of 21.5 and expression of IL-1β is increased in untreated mice relative to Cef & iAs treated sick mice by a multiplicative factor of 12.2. Compact letter displays at the top of the charts illustrate significant ($p < .05$) pairwise differences between groups per Tukey's Honestly Significant Different (HSD) method based on ANOVA. These box plots depict the median, upper, and lower quartiles, with whiskers extending to 1.5 times the interquartile range. Dots represent individual samples.

CONCLUSIONS & FUTURE DIRECTIONS

- Blood and sera analysis show that sick mice co-exposed to iAs & Cef exhibit organ dysfunction and a dysregulated host immune response, both of which are indicative of sepsis.⁵
- Analysis of liver cytokine expression shows there are no significant differences between sick and healthy mice co-exposed to Cef and iAs. Notably, we show sick mice had significantly lower expression of IL-1B compared to untreated mice. These inconclusive results may be due to the fact the liver is not an immune organ. Future work will aim to assess cytokine expression in immune tissues like the thyroid.
- Analysis of cecum aquaporin expression shows there are no significant differences between sick and healthy mice co-exposed to Cef and iAs. However, we show sick mice had significantly lower expression of aqp8 compared to untreated animals.
- Future work will employ culture-dependent and independent methods to find evidence of bacterial infection in the blood and organs of sick mice co-exposed to Cef & iAs.
- Future work will also investigate what specific immune cell populations are lost in sick mice compared to healthy mice co-exposed to Cef & iAs.

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REFERENCES

