



# The comparison of preservation and DNA extraction methods for the molecular detection and quantification of soil-transmitted helminth infection in stool

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## Background

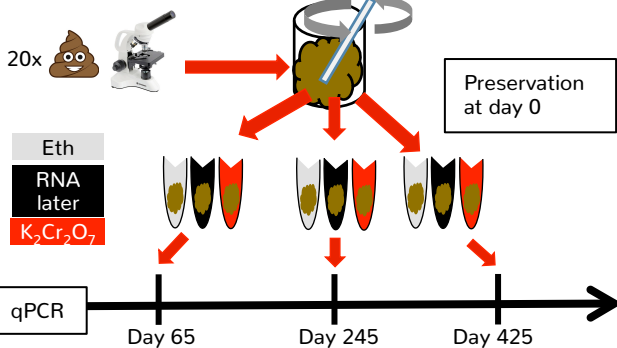
- The current means to diagnose soil-transmitted helminths (STH) infections is based on the microscopic detection and quantification of eggs in stool
- Nucleic acid tests have important advantages
  - ✓ increased sensitivity
  - ✓ simultaneous detection of other pathogens
  - ✓ differentiation of STH species
  - ✓ early detection of anthelmintic resistance
- However, it remains unclear
  - ✓ how and how long stool can be preserved prior analysis
  - ✓ how the DNA yield can be maximized

## Aims of the study

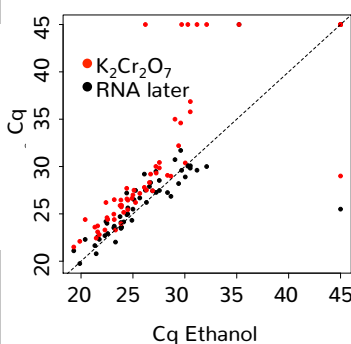
- Compare the DNA yield for 3 preservatives over time
  - ✓ 100% ethanol (Eth)
  - ✓ RNA later
  - ✓ 5% potassium dichromate (K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>)
- Compare the DNA yield for 4 DNA extraction methods
  - ✓ QIAamp mini stool kit (SK)
  - ✓ QIAamp mini stool kit + bead beating (SK+B)
  - ✓ DNeasy blood & tissue kit (TK)
  - ✓ DNeasy blood & tissue kit + bead beating (TK+B)

## Preservation of stool

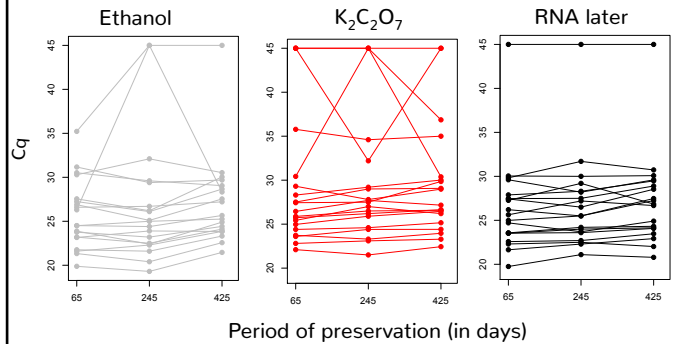
### Study design



### Results



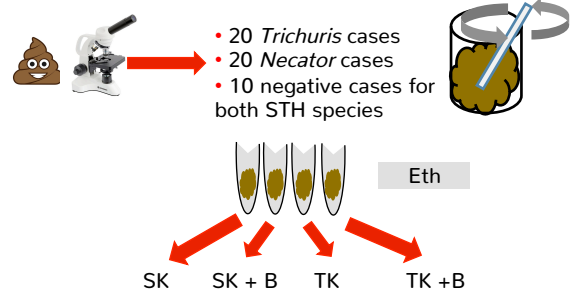
- Figure illustrates the agreement in Cq for 3 preservatives for *Trichuris*
  - Preservation in ethanol and RNA later resulted in comparable Cq values
  - Preservation in K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> resulted in higher Cq values
- Similar results were found for *Ascaris* and *Necator* (not shown)



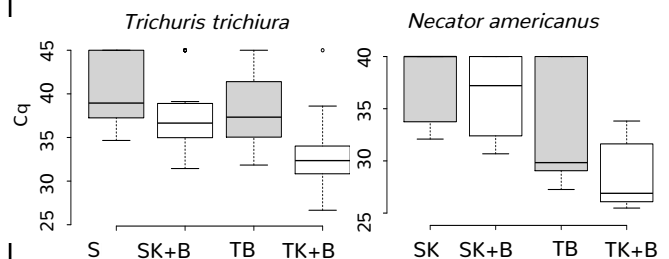
- As illustrated for *Trichuris* in the Figures above, DNA yield remained stable over time for the 3 preservatives
- Similar results were observed for *Ascaris* and *Necator*

## DNA extraction

### Study design



### Results



- Bead-beating increases DNA yield
- DNeasy blood & tissue kit higher DNA yields compared to QIAamp stool kit

## Conclusions

- Absolute ethanol** is the most cost-effective preservative
  - ✓ cheap and available in STH endemic countries
  - ✓ storage up to 14 months has no impact on DNA yield
- DNeasy blood & tissue kit + bead beating** is the most cost-effective DNA extraction procedure
  - ✓ maximizes the DNA yield
  - ✓ kit is cheaper and less labor intensive than QIAamp