Mycobacterium avium paratuberculosis in Crohn's:
a study using tissue PCR, *in situ* PCR and interferon-γ responses

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Background

- M. *avium paratuberculosis* (MAP): emerging pathogen, cause of IBD in cattle and other animals
- Histopathological similarities between certain types of paratuberculosis (sheep paucibacillary) and Crohn's disease
- Issues in proving link between MAP and Crohn's:
  - Technical: accurate diagnostic tests
  - Conceptual: study design
    - Case definition/classification of Crohn's disease
    - Appropriate controls

Hypothesis

- Humans are exposed to MAP
- A subset of exposure leads to infection
- A subset of infection presents as IBD
  - Either Crohn's disease or Crohn's-like disease
  - Conversely, all Crohn's not necessarily due to MAP

Objectives

- Detect MAP DNA in tissue samples of excisional biopsies from Crohn's and controls:
  - tissue PCR
  - *in situ* PCR
- Determine prevalence of immunologic reactivity to MAP in Crohn's and controls:
  - cell-mediated immune response (IFN-γ assay) to Mycobacterial antigens (MAP and others)

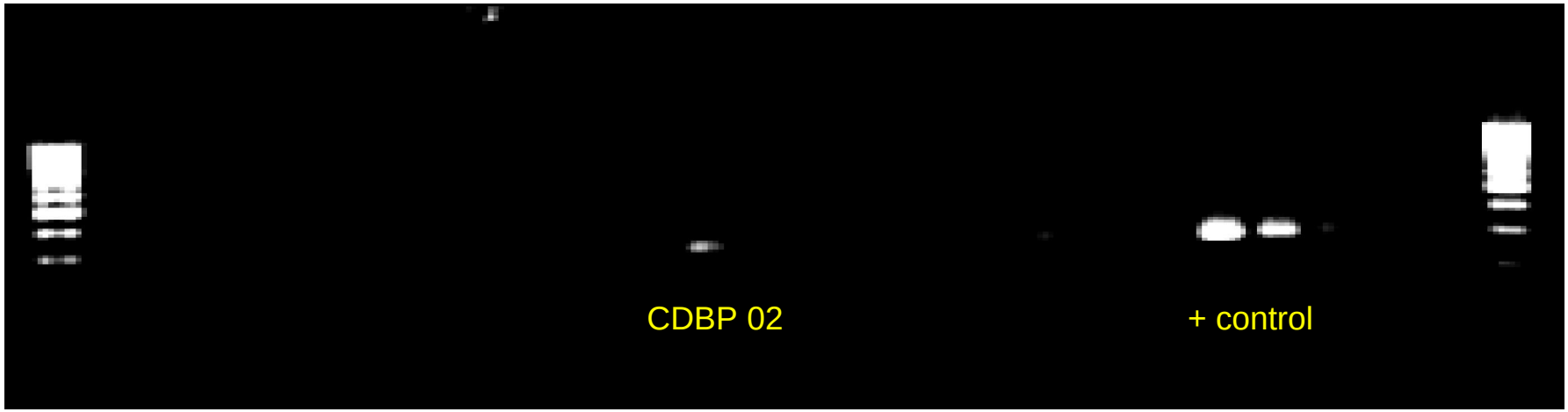
Techniques used: MAP DNA

- Tissue PCR (IS*900* in extracted DNA):
  - Paucibacillary paratuberculosis samples from sheep amplify for MAP and not TB
    - Sensitive: 12/12, acid-fast negative samples (<10,000 bact/mL)
    - Specific: IS*900* does not amplify in TB samples
- *in situ* PCR (IS*900* on pathology slide):
  - Sheep samples and BCG infected mouse tissue
    - Sensitivity: improved detection versus *in situ* hybridization
    - Specificity: again – MAP amplifies from paratuberculosis only

Techniques used: immune studies

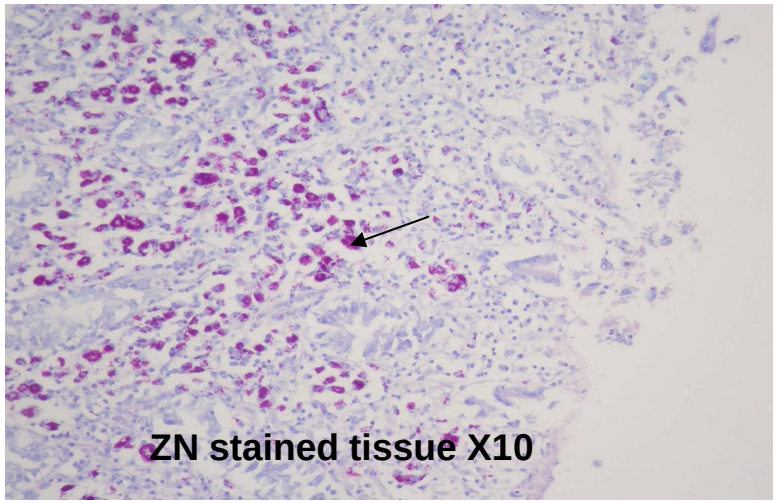
- IFN-γ assay:
  - PBMCs from study subjects stimulated *in vitro:*
    - PHA (positive control)
    - MAP tuberculin (paratuberculin)
    - Other Mycobacterial antigens
  - IFN-γ expression measured by Q-RT-PCR

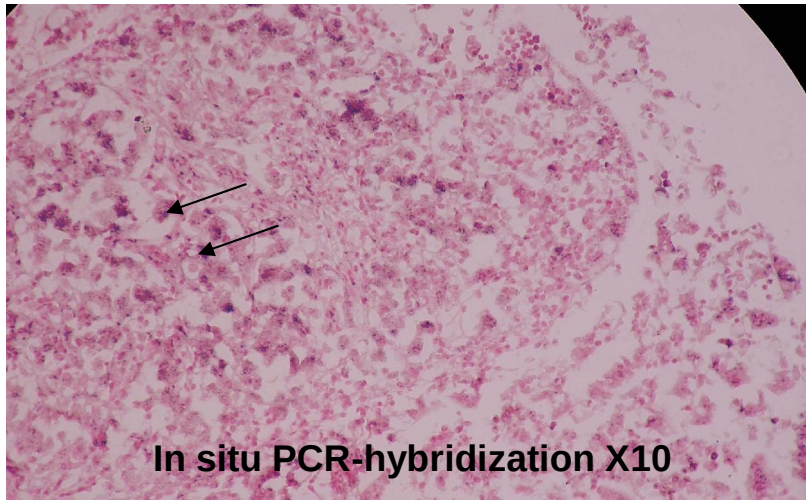
Tissue PCR: First 14 samples

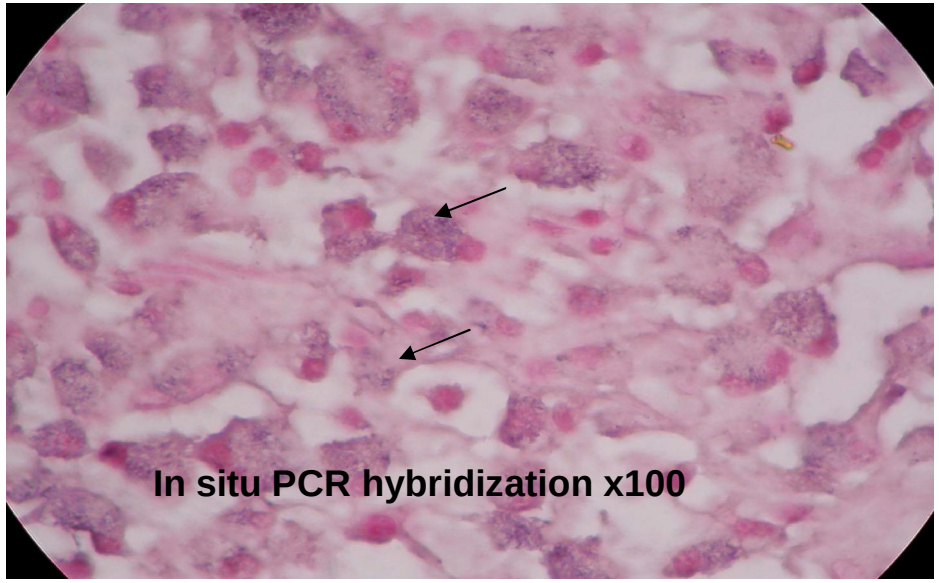


IS*900* for M. paratuberculosis (1/14)

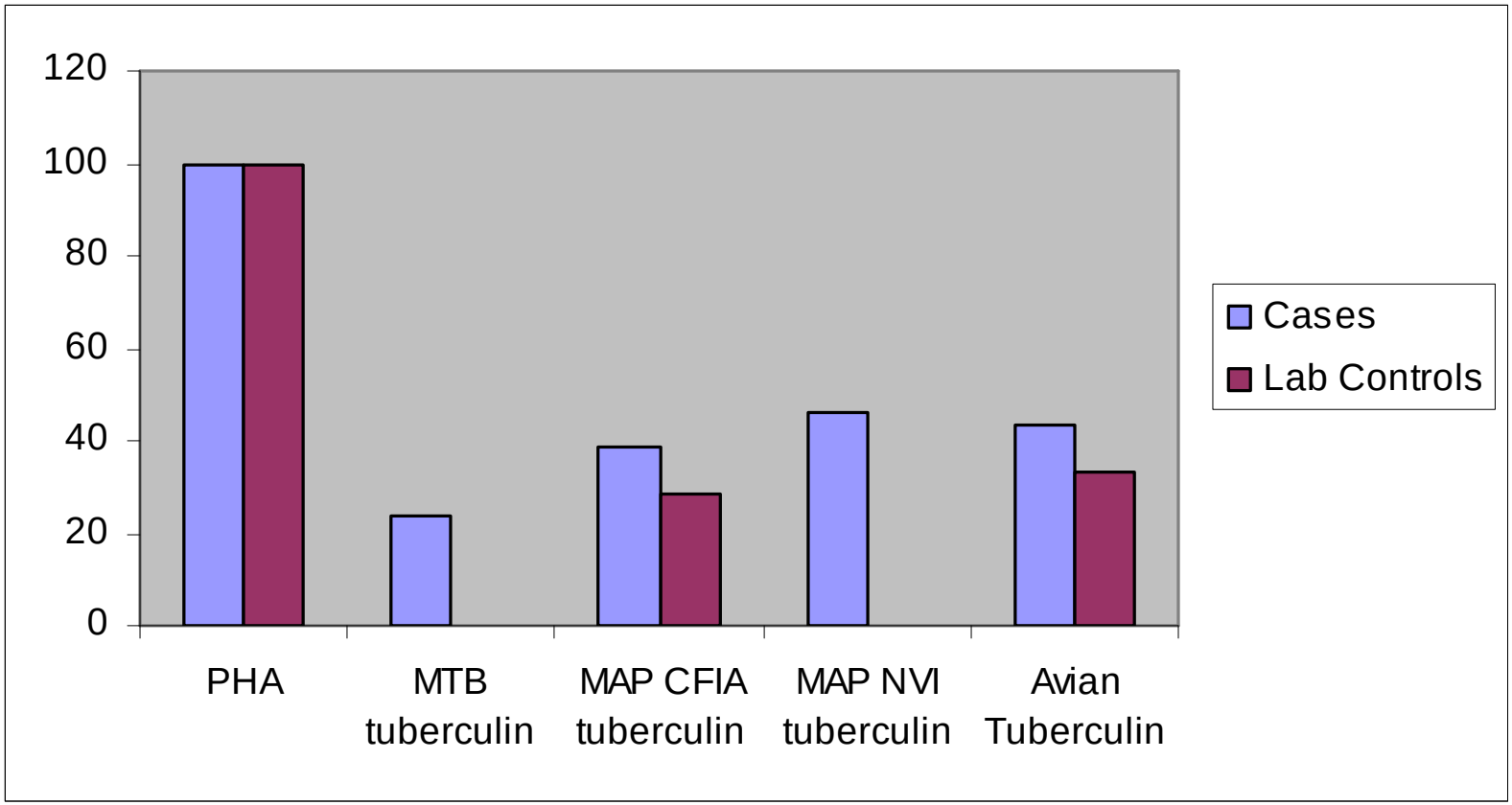
In situ PCR: Sheep paratuberculosis







IFN-γ assay: First 60 samples



Provisional interpretation

- PCR: too early to judge
- In situ PCR: Promising modality, utility remains to be seen (stand-alone?, confirmatory?)
- Mycobacterial immunity: Exposure to *Mycobacterium avium* complex clearly common
- Cases vs. controls: Assays blinded to subject classification, do not know if any trends exist

Upcoming directions

- PCR: to complete ~100 by July 2005
- In situ PCR: to complete by summer 2005
- Mycobacterial immunity: Already studied ~60, to complete by May 2005
- Unblinding and analysis: summer 2005
- Blood stored on subjects for humoral studies and genetic analysis in the future