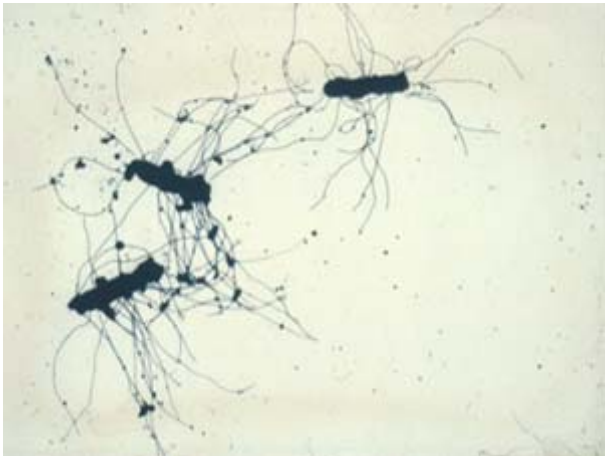


# *Clostridium difficile*: an overview of the diseases it causes, host defences, risk factors and changing host susceptibility



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Chair ESCMID European Study Group  
for *Clostridium difficile***



# Plan of talk

- The disease
- Who gets CDI?
- Risk factors
- Are patterns changing?
- Pathogenesis
- Host defences
- Conclusions/questions



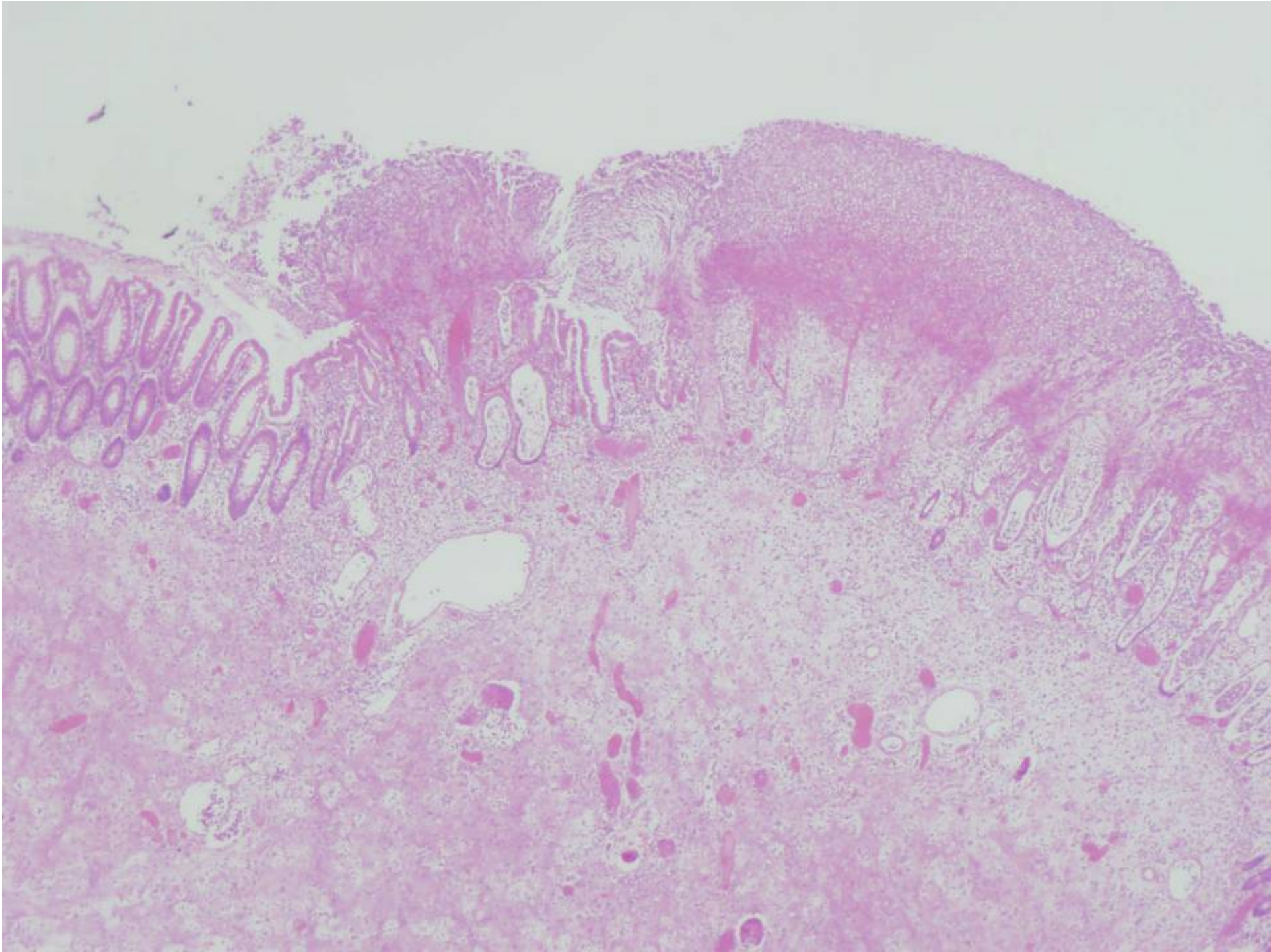
# The Disease

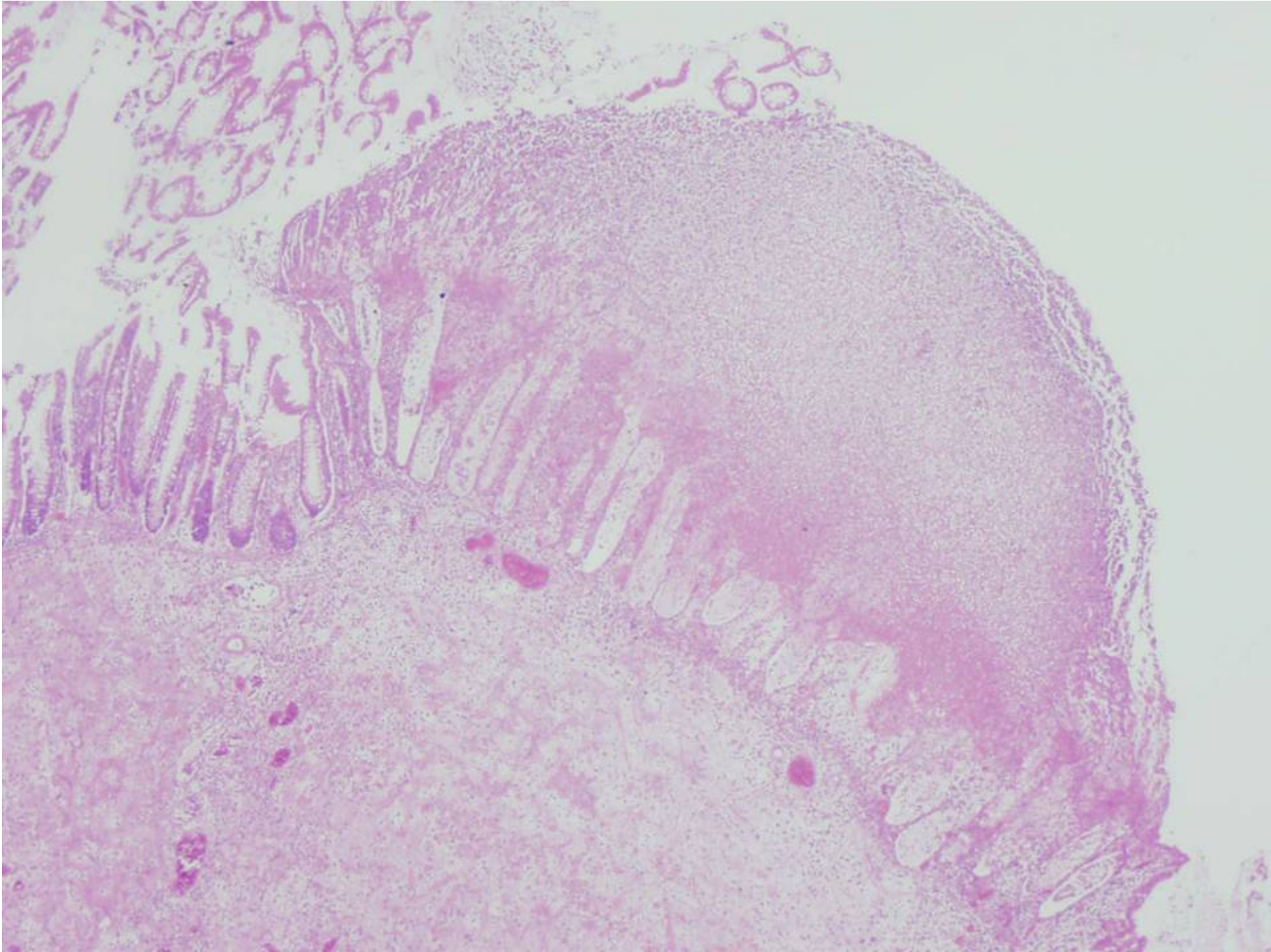
## *Clostridium difficile* infection (CDI)

- A spectrum of severity
  - Mild diarrhoea, severe diarrhoea, pseudomembranous colitis to life-threatening fulminant colitis
- Consensus that it has been increasing and there's certainly increasing awareness
- Unravelling facts from impressions...

# Pseudomembranous colitis









# Who gets CDI?

## Local findings 2000-2007

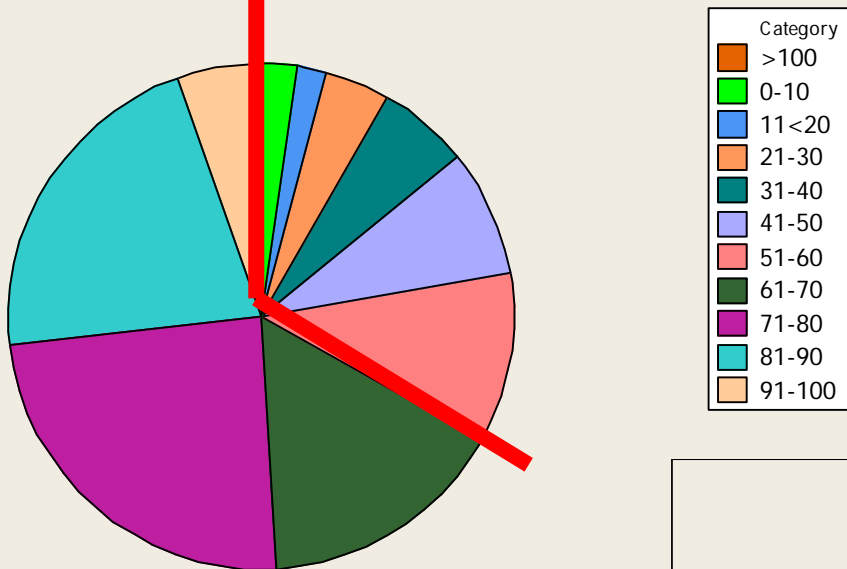
Retrospective analysis of all specimens (almost 50000) that were submitted for *C. difficile* toxin testing

- Age of patient
- Clinical specialty

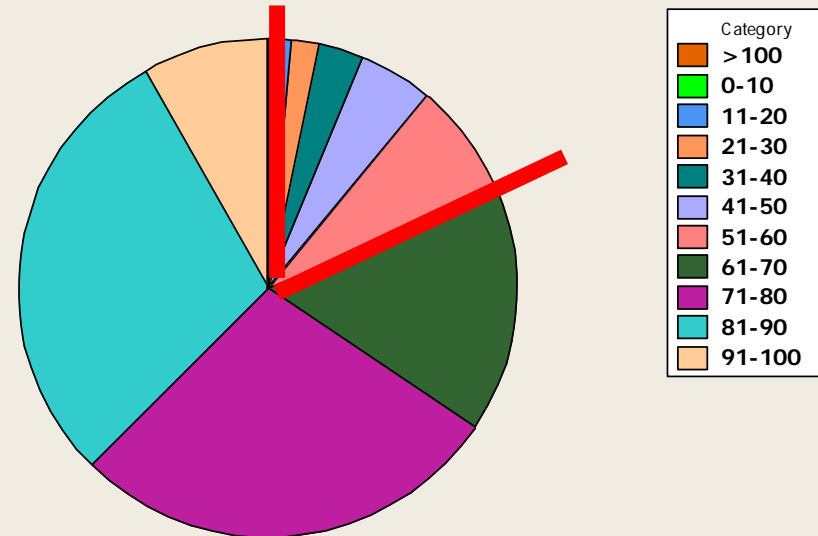
# AGE DISTRIBUTION



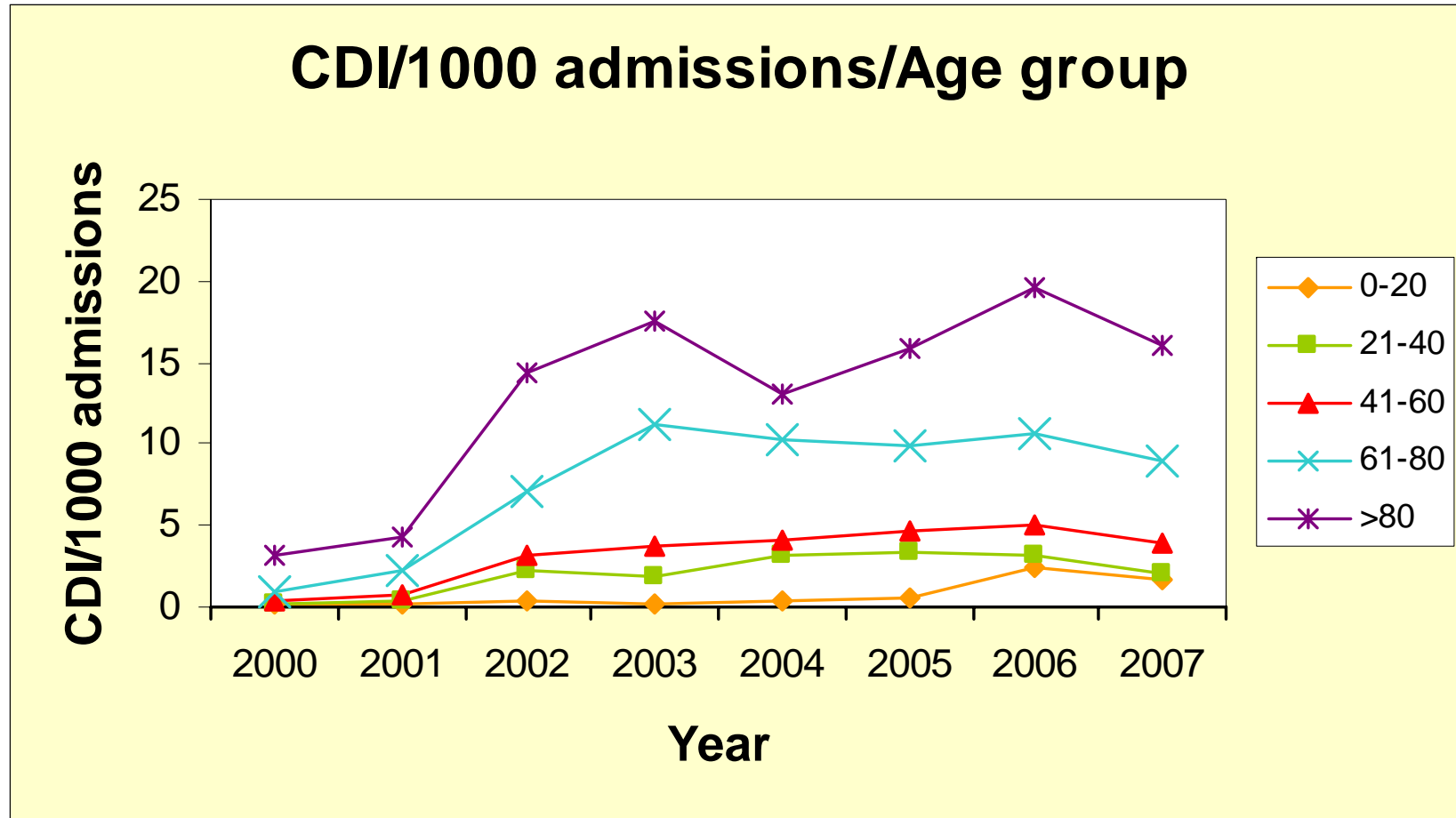
All samples



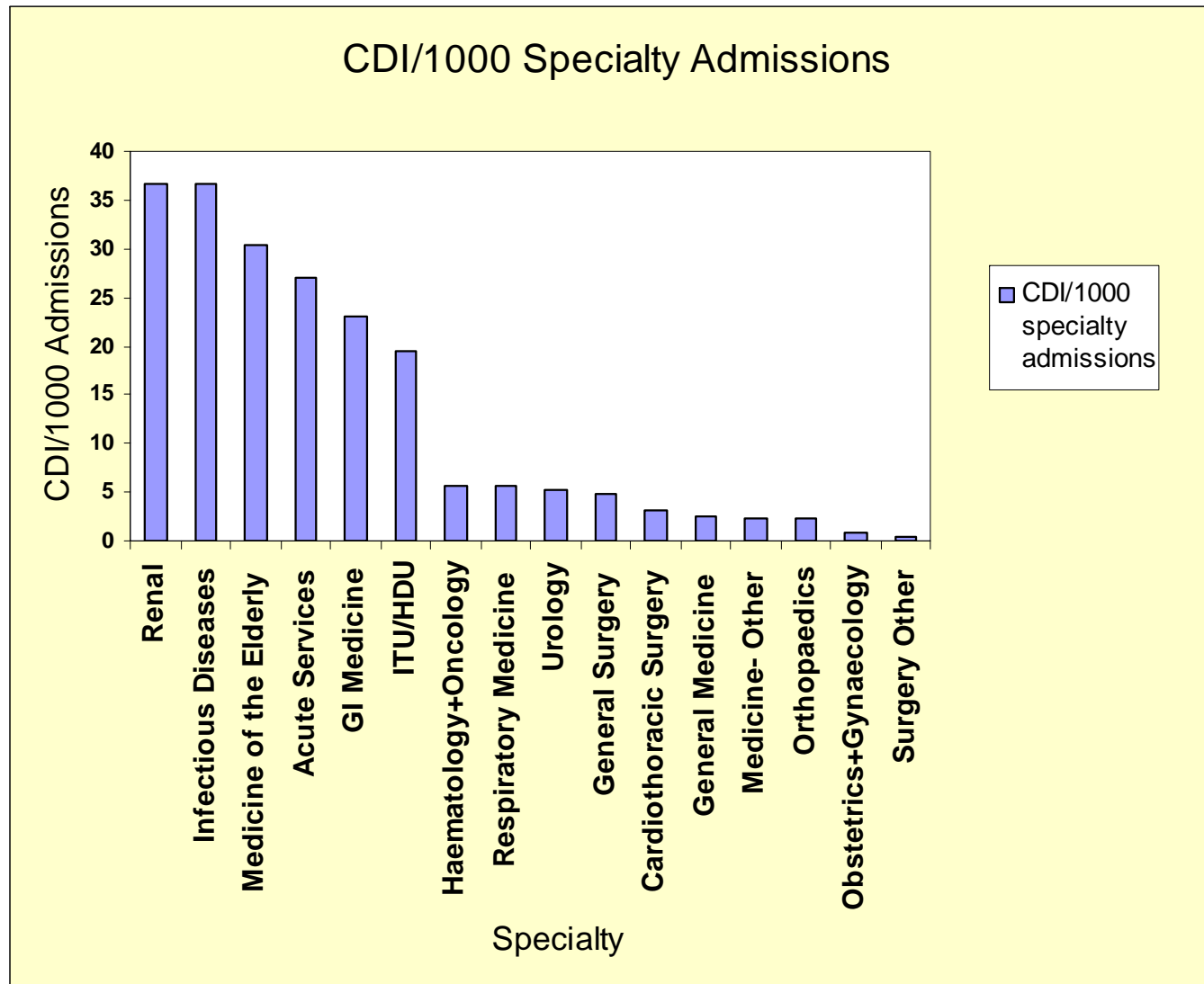
Positive samples



# AGE DISTRIBUTION



# Which patients develop CDI





# Risk Factors for CDI: The Common View

- Being elderly
- Being treated with antibiotics
- Being in hospital

# Additional Risk Factors



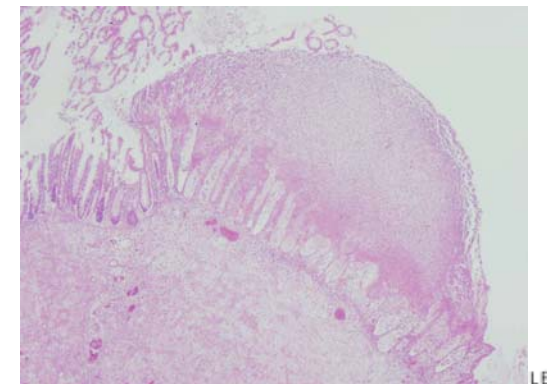
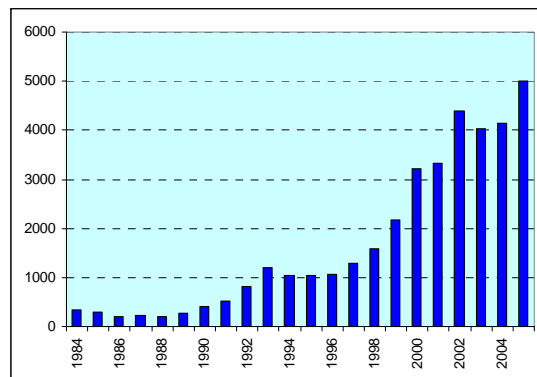
- Increasing age (excluding infancy?)
- Severe underlying disease
- Non-surgical gastrointestinal procedures
- Presence of a naso-gastric tube
- Receiving anti-ulcer medications
- Stay on intensive care unit
- Long duration of hospital stay
- Long duration of antibiotic course
- Receiving multiple antibiotics
- Receiving specific antibiotics
  - Clindamycin, cephalosporins and penicillins
- Immune status of patient

**Their relative importance???**

# CDI: Some Recent Facts and Perceptions Worldwide



- Number of cases and severity of disease have increased
- Treatment is more difficult by traditional methods
- Rate of relapse/recurrence has increased
- More disease in "unusual host groups"
  - in the community in seemingly healthy, non-institutionalised individuals
  - in younger patients
  - in peripartum women
  - In those not apparently exposed to antimicrobial agents
- Associations with other GI pathogens (e.g. norovirus)
- Possible zoonotic route of acquisition
- **More virulent strains of *C. difficile* have been reported**



# But Is Anything New?



## Our study in 1982

- 19 (12%) of 154 of patients admitted from the **community** to an infectious disease unit with sporadic serious diarrhoea harboured *C. difficile*
- 14 had neutralisable toxin in stools
- 4 had co-infection with *Salmonella* spp
- 2 had no antibiotic history

# Early (Sometimes Overlooked/ Forgotten) Observations



- Co-infection with other GI pathogen(s)
  - Falsen E, et al. *J Clin Microbiol.* [1980](#); 12:297-300
  - Brette RP, et al. *BMJ.* [1982](#);284:230-233
- Community associated
  - Brette RP, et al. *BMJ.* [1982](#);284:230-233
- Infected by multiple types
  - Sharp J & Poxton IR. *J Immunol Methods* [1985](#);83:241-248.
- Relapse caused by both re-infection and re-acquisition of new strain
  - McKay I, Coia JE, Poxton IR [1989](#); *J Clin Path* 42: 511-515
- Common in the environment
  - Al-Saif NM, Brazier JS. *J Med Microbiol.* [1996](#);45:133-137
- Severity of underlying disease is an important risk factor
  - Kyne L, et al. *Infect Control Hosp Epidemiol.* [2002](#);23:653-659



# A Changing Picture...

## Recognition of O27/NAP1/BI

- Major outbreaks in North America and Western Europe
- More severe disease
- More deaths
- Increased level of toxin production
- Resistance to fluoroquinolones
- Binary toxin positive

**A new hypervirulent strain  
has emerged**

# An unusual case history



# 23 year-old female

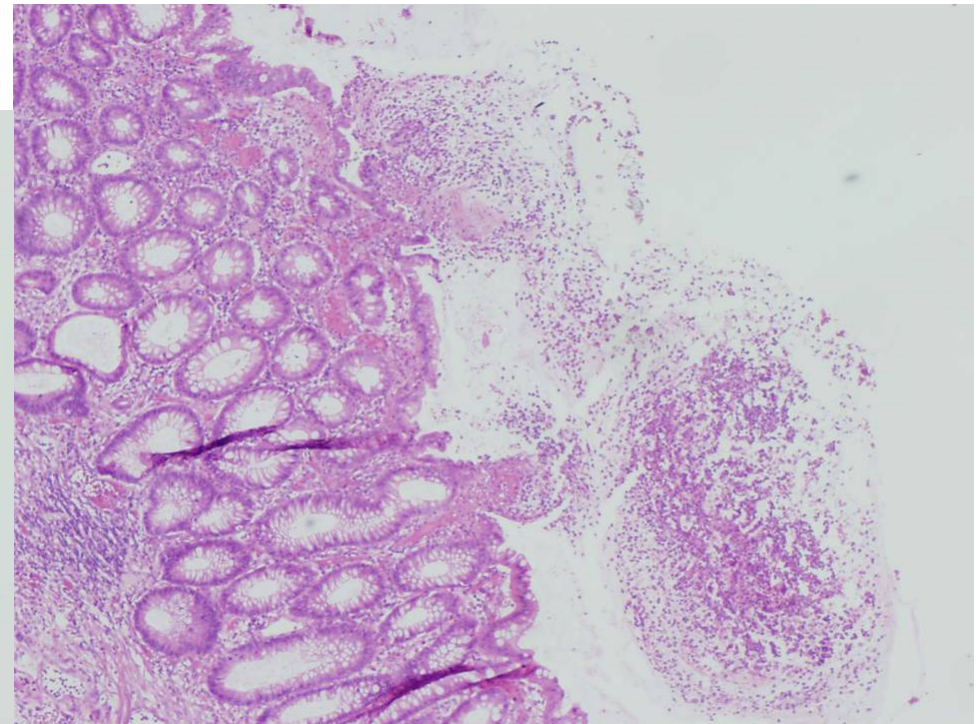
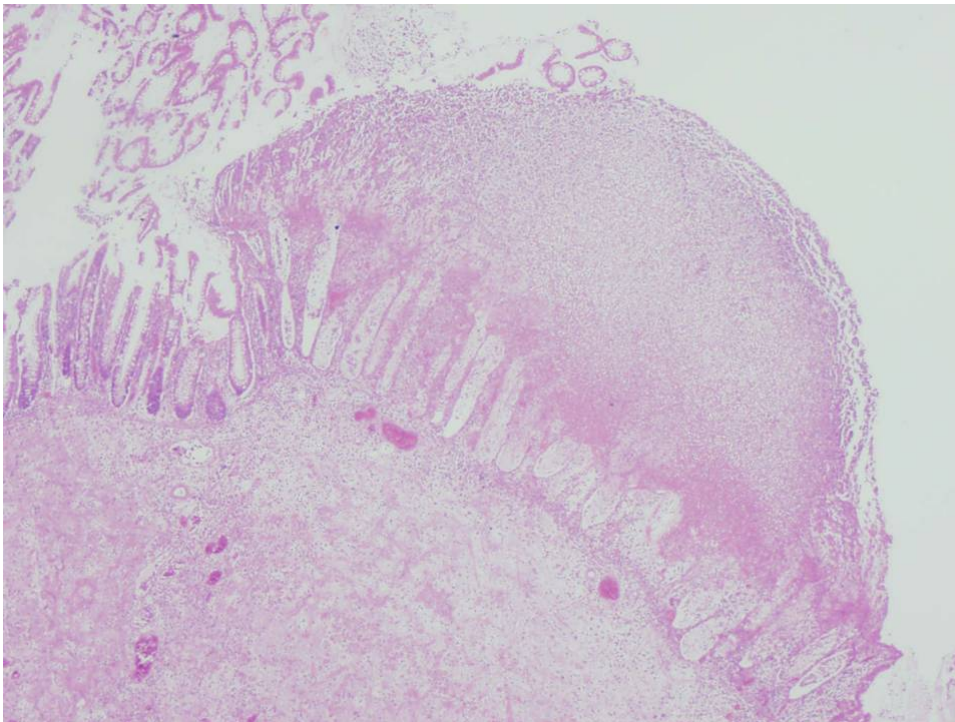


- Healthy rugby player - broke femur
- Developed lipid embolism in lung
- Required ventilatory assistance
- Developed VAP
- Treated with multiple including 3<sup>rd</sup> gen cephalosporin
- Developed severe PMC/toxic megacolon
- Required surgical resection to save life
- Eventually recovered



# Moral of tale:

Girls shouldn't play rugby



# Pathogen versus host?

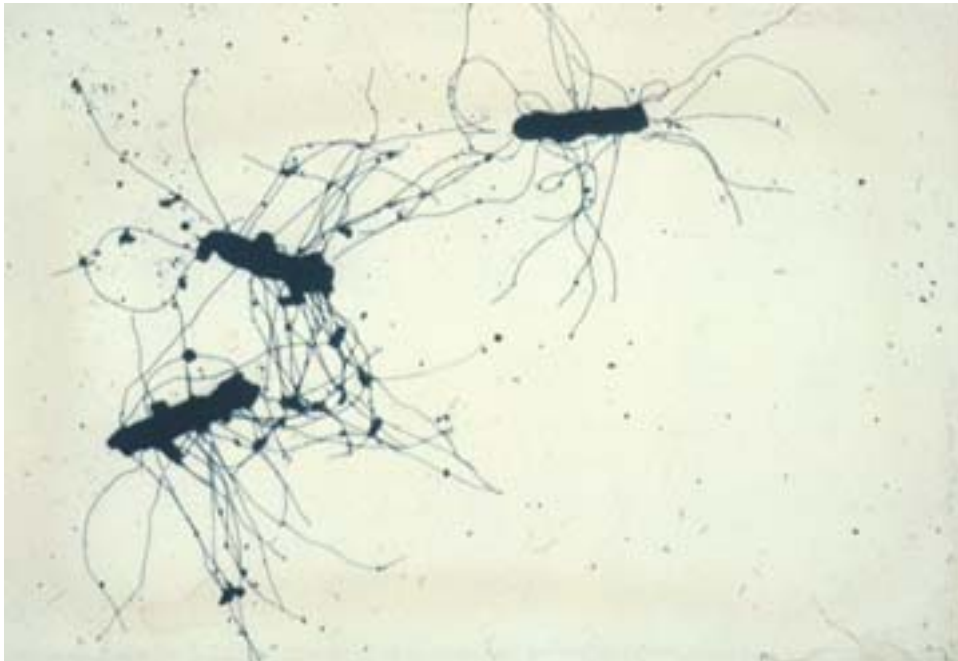
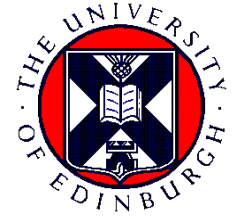


Following acquisition of bacterial spores and subsequent colonisation by the bacterium

Outcome is dependent on both

- the virulence of the organism and
- the susceptibility of the host.

# The pathogen



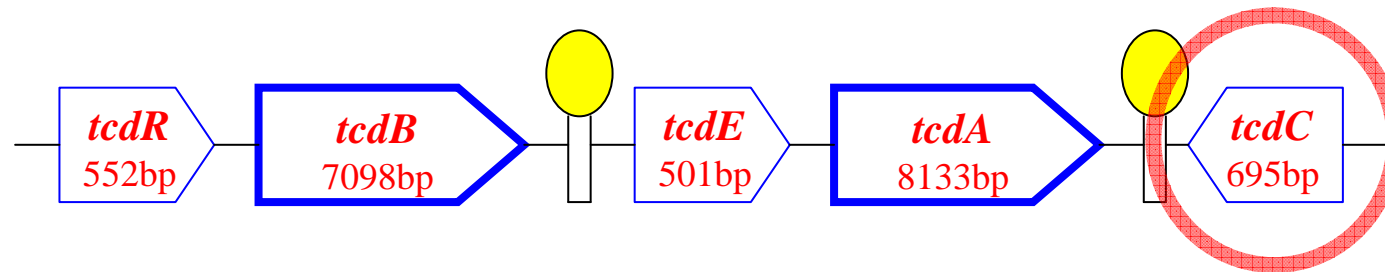
- Gram-positive
- Anaerobic
- Spore former
- Motile
- Increasingly resistant to antibiotics



# Pathogenesis of CDI

## Toxins A and B

# Pathogenicity Locus (PaLoc)



- *tcdR* alternative sigma factor - positive regulator of toxin production
- *tcdC* negative regulator
- *tcdE* encodes a holin-like protein
- toxins transcribed on entry to stationary phase

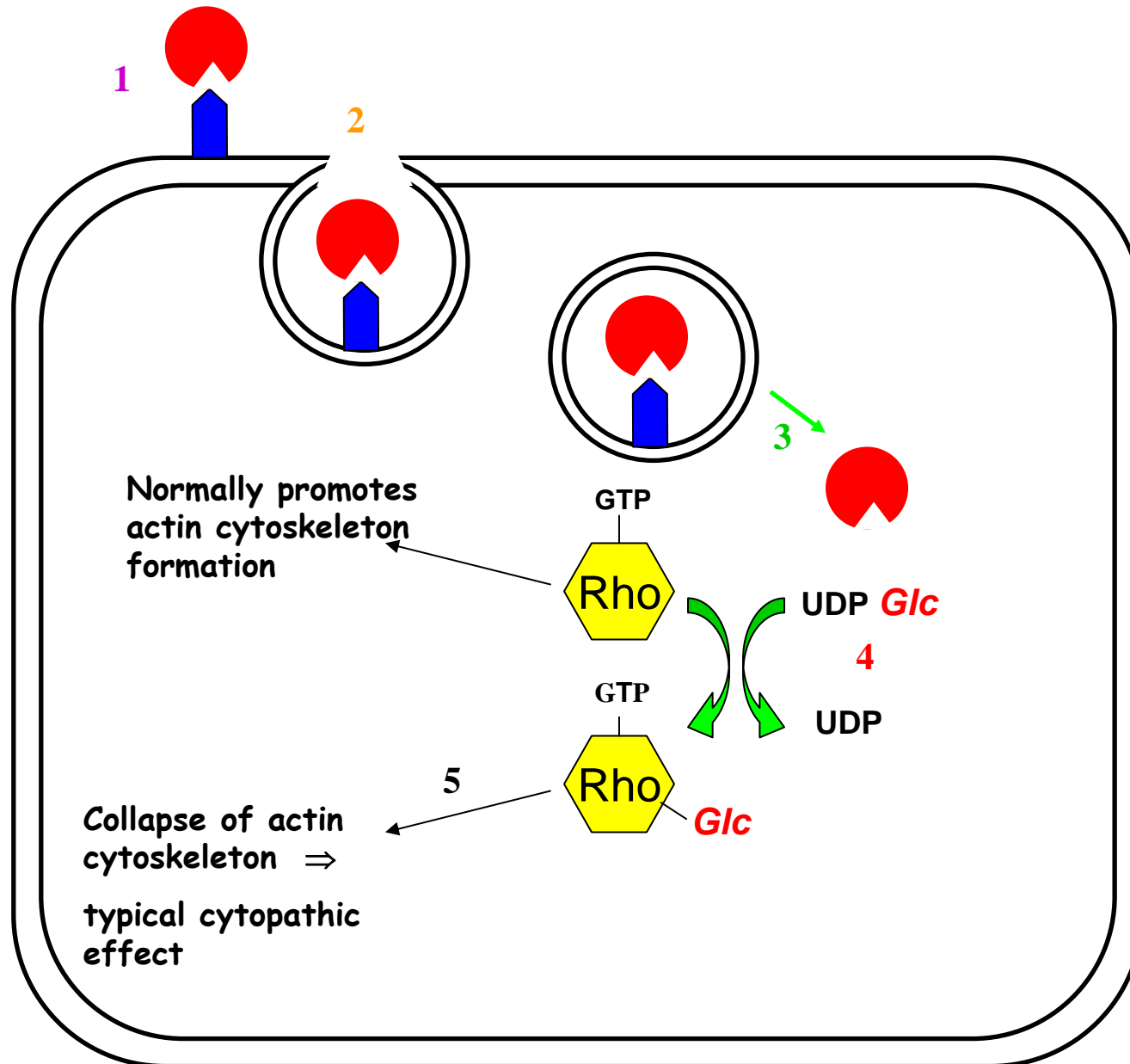
# Pathogenesis: Host-pathogen relationships



The symptoms of the disease are a result of one or both of the two major virulence factors of the organism (toxins A and B or B alone) exerting their effects on the colonic mucosa.

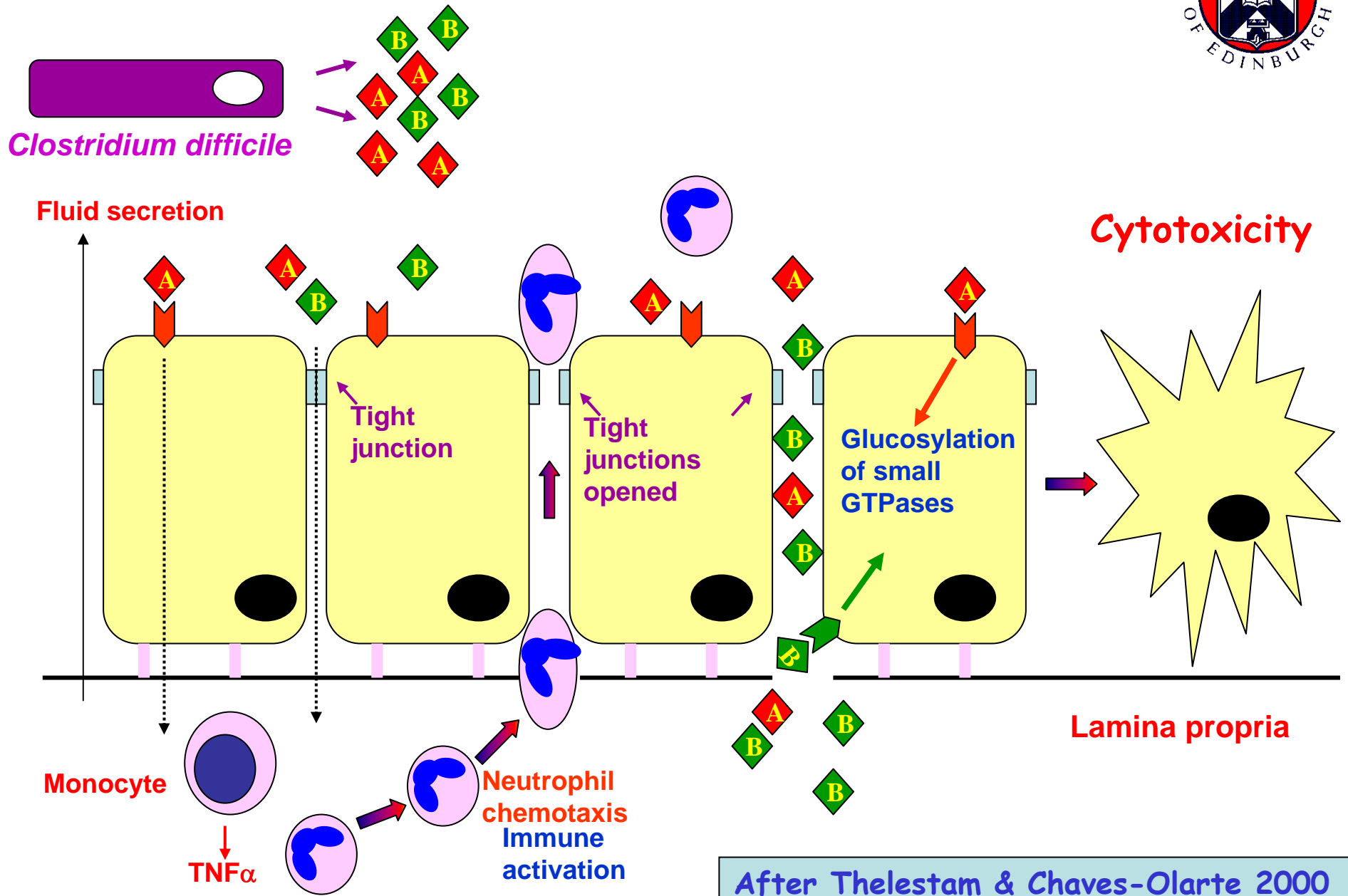
[contribution of other virulence factors - including binary toxin are unknown]

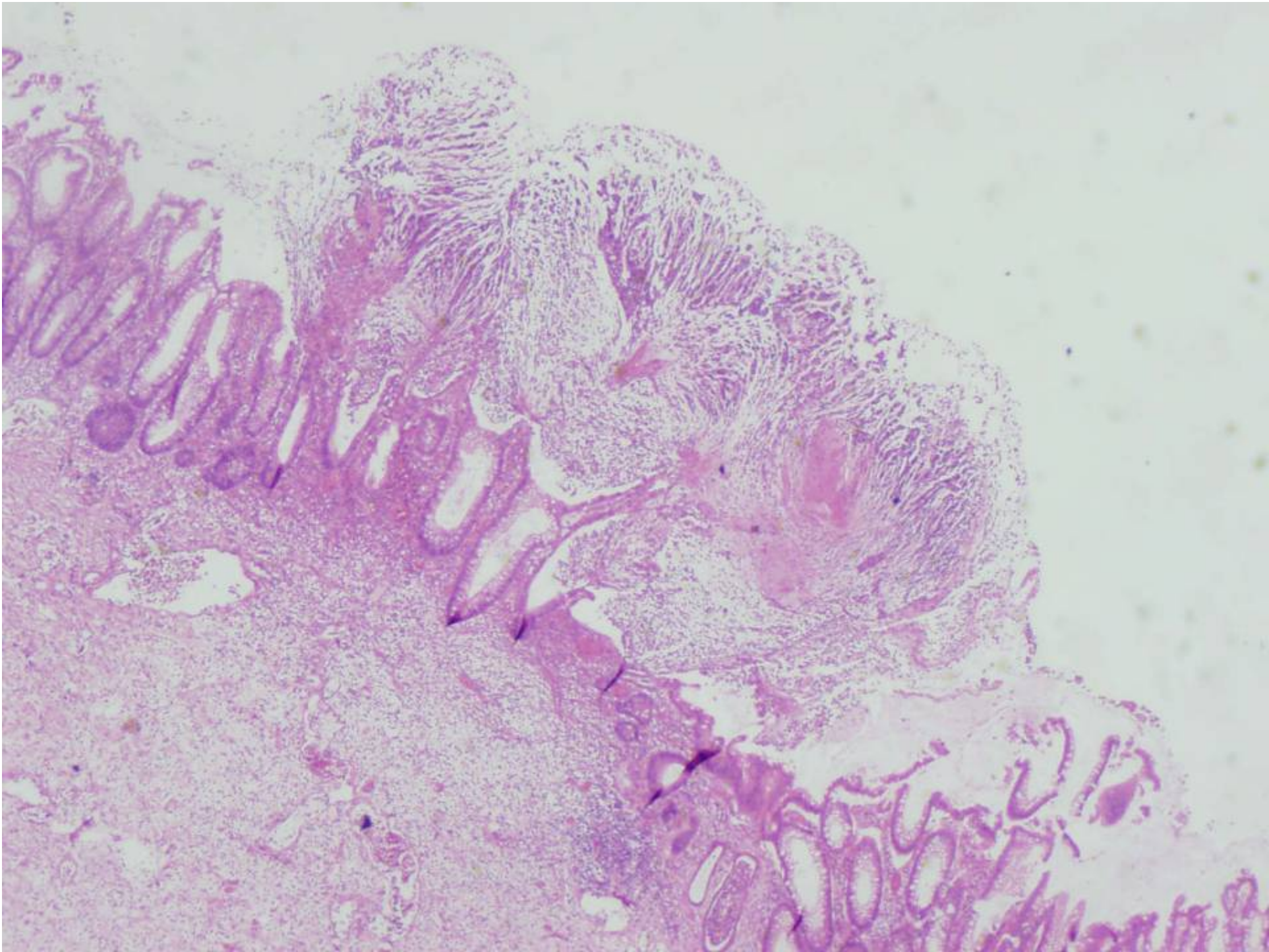
# Simplified scheme for the action of *C. difficile* toxins on cells



1. Toxin binds to specific receptor (Gal  $\beta$ 1-4GlcNAc for A)
2. Toxin enters cell by receptor-mediated endocytosis
3. Translocation from endosome/lysosome?
4. Rho and other small GTP-binding proteins are glucosylated
5. Glucosylated GTPases cause collapse of actin cytoskeleton - rounding with neurite-like protrusions remaining

# Actions of *C. difficile* Toxins A and B on intestinal epithelium





# Two major mechanisms of damage to the host



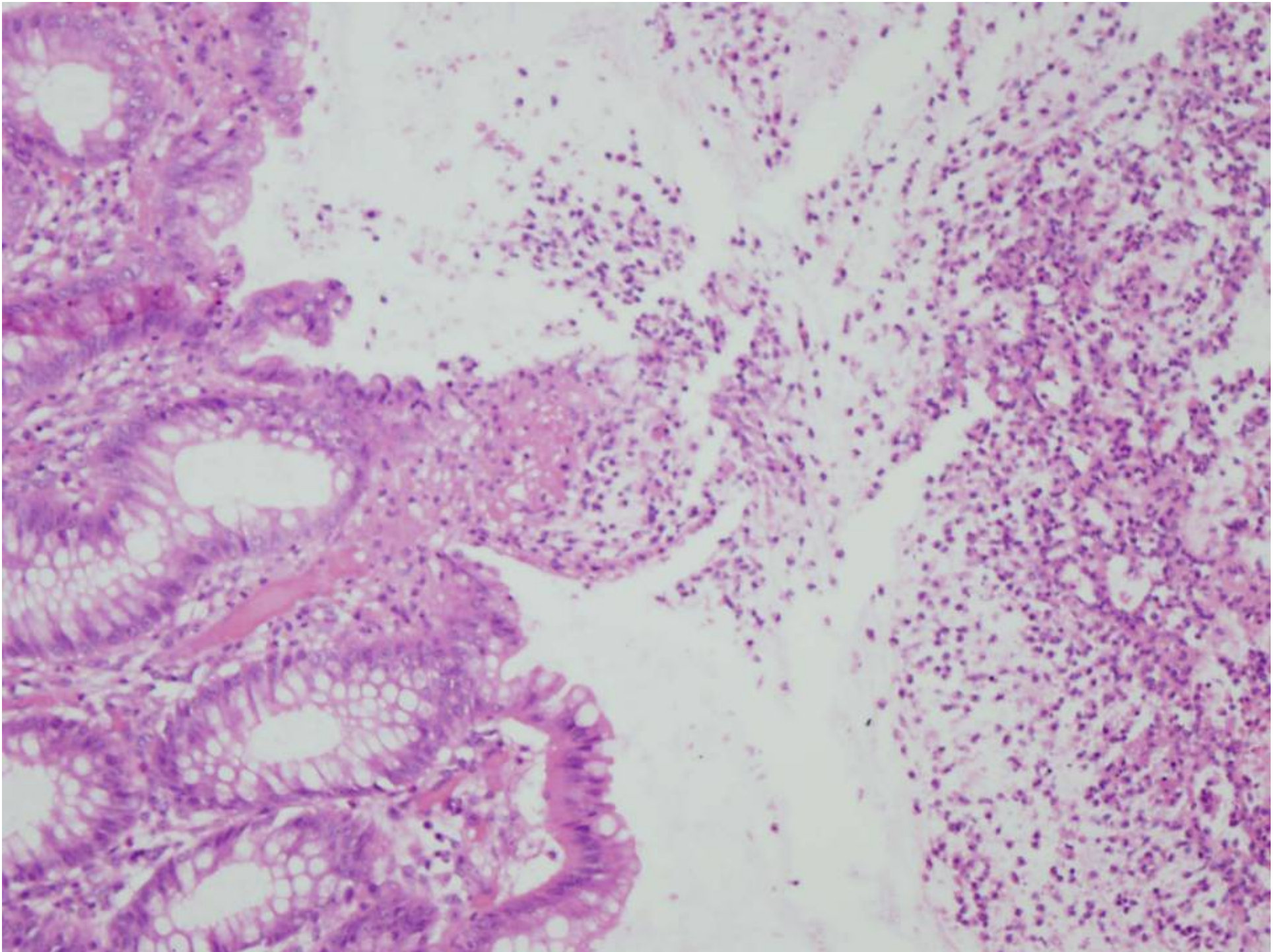
- i) The cytotoxic properties of the toxins damage the colonocytes (cytopathic effect) and
- ii) The action of the toxins in the lamina propria result in a possibly intense inflammatory response
  - induction of pro-inflammatory cytokines
  - recruitment of neutrophils
  - + fibrin → pseudomembrane formation.

# Consequences of damage



These combined actions result in

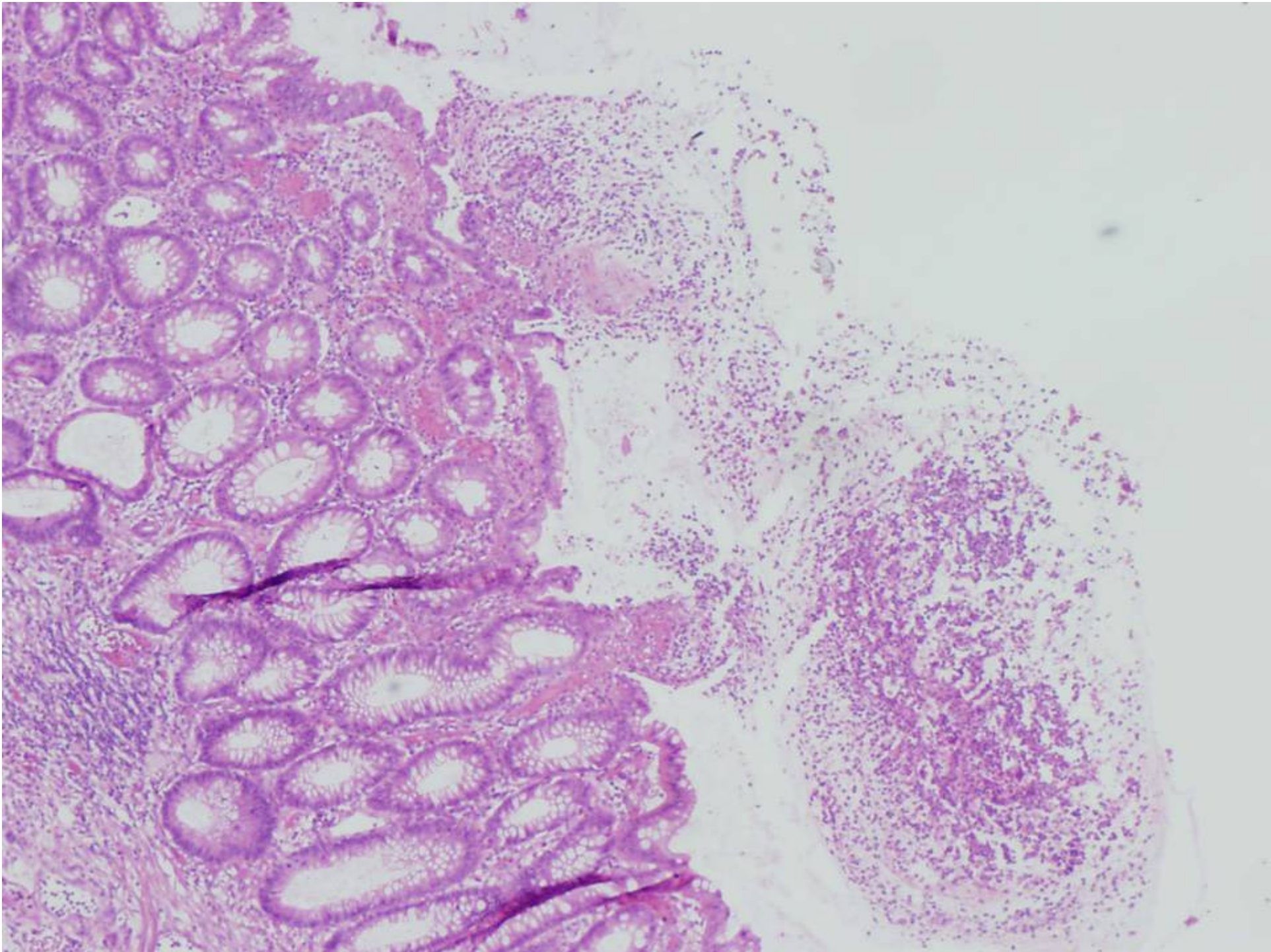
- the loss of fluid by leakage through the damaged mucosa
- reduced absorption
- the formation of pseudomembranes
  - following the "explosion" of neutrophils through the damaged mucosa and opened tight junctions with accumulations of fibrin.
- General gut inflammation, toxic megacolon, risk of perforation



# Severity of symptoms



- Symptoms are largely independent of the strain causing the disease?
- The same strain can bring about different effects in each infected host.
- Although uncommon in most healthy individuals, *C. difficile* is carried asymptotically by many hospitalised elderly as well as the neonate.



# But... significant bacterial factors include



- the amount and rate of production of the toxins
- the infectivity of the bacterium
- its degree of sporulation and spore survival inside and outside the host

Consider ribotype 027 strains

- thought to be hypervirulent because of increased toxin production.
- a result of a deletion in their *tcdC* gene - the negative regulator of toxin transcription.
- Similar deletions are found in other potentially virulent strains e.g. 078 as well as some not currently recognised as being hypervirulent.



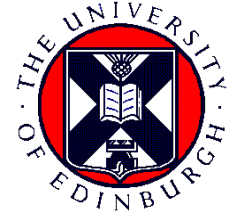
# The host

The neglected (**difficult**) side of the subject

What do we know and what do we not know?

A tale of ethical and logistical problems!

# Patient susceptibility



## Major risk factors?

- The degree of disruption of the normally protective colonic microbiota and subsequent loss of colonisation resistance
- Following the action of
  - antibiotics
  - other agents that might affect the gut ecosystem,
  - including co-infection with another GI pathogen
- Other factors might include level of gastric acid and gut motility

# Host response and susceptibility



- The lack or inappropriateness of **local** and **systemic antibodies** to the virulence factors of the organism
- The degree and specificity of the various **cellular responses**.
- Influenced by
  - the severity of underlying disease,
  - the degree of immunocompromisation
  - immunosenescence
  - genetic polymorphisms in response genes (e.g. IL-8, TNF-alpha).

# Our current studies on host susceptibility



- Systemic antibodies
- Cellular mucosal responses
- Immunosenescence
- Host polymorphisms



# Patient groups

- **Cases:** *C. difficile* toxin and culture-positive and **symptomatic**
- **Carriers:** *C. difficile* culture-positive **asymptomatic**, toxin variable
- **Controls:** age, sex and ward matched ***C. difficile*-negative and asymptomatic**

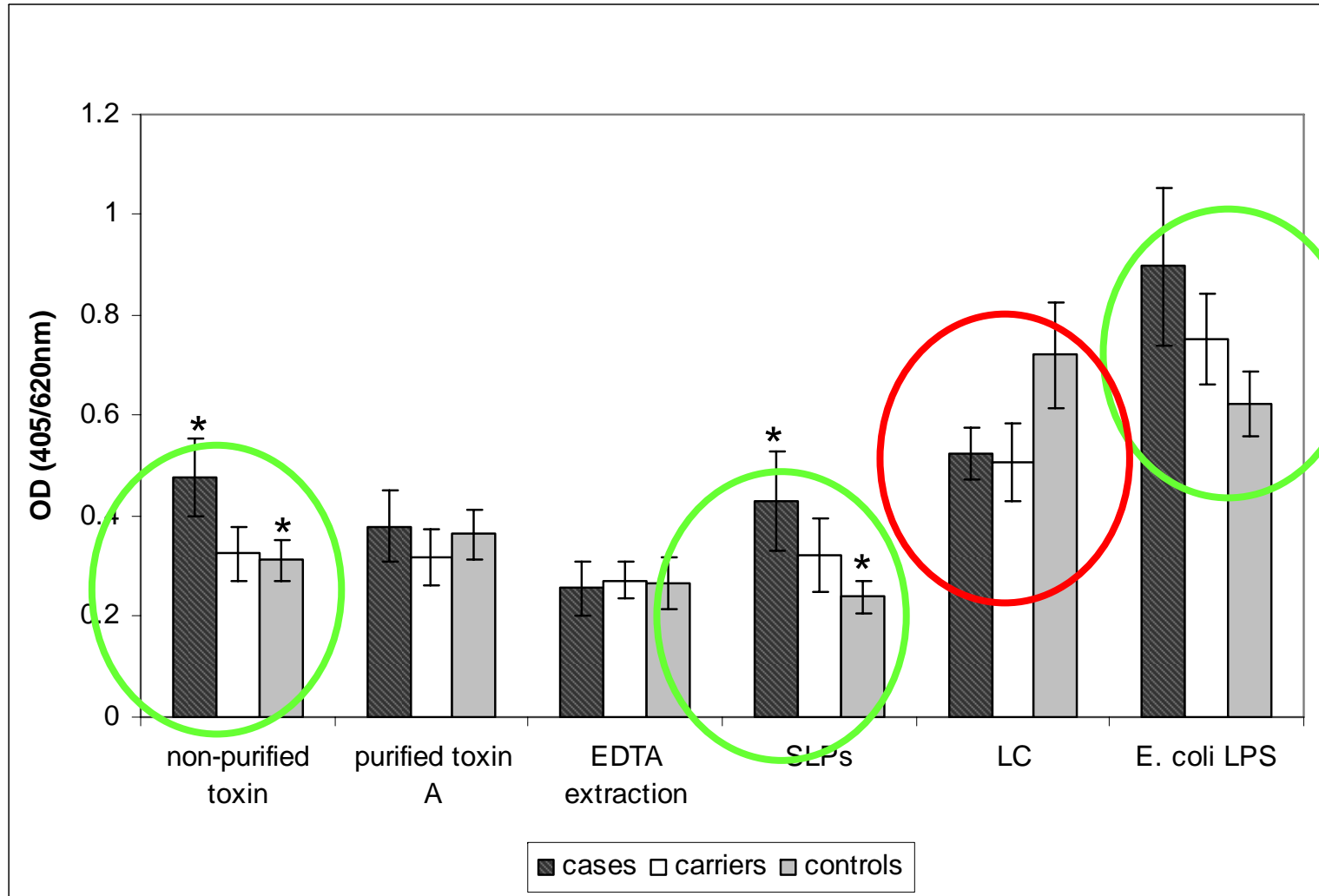


# Systemic antibodies

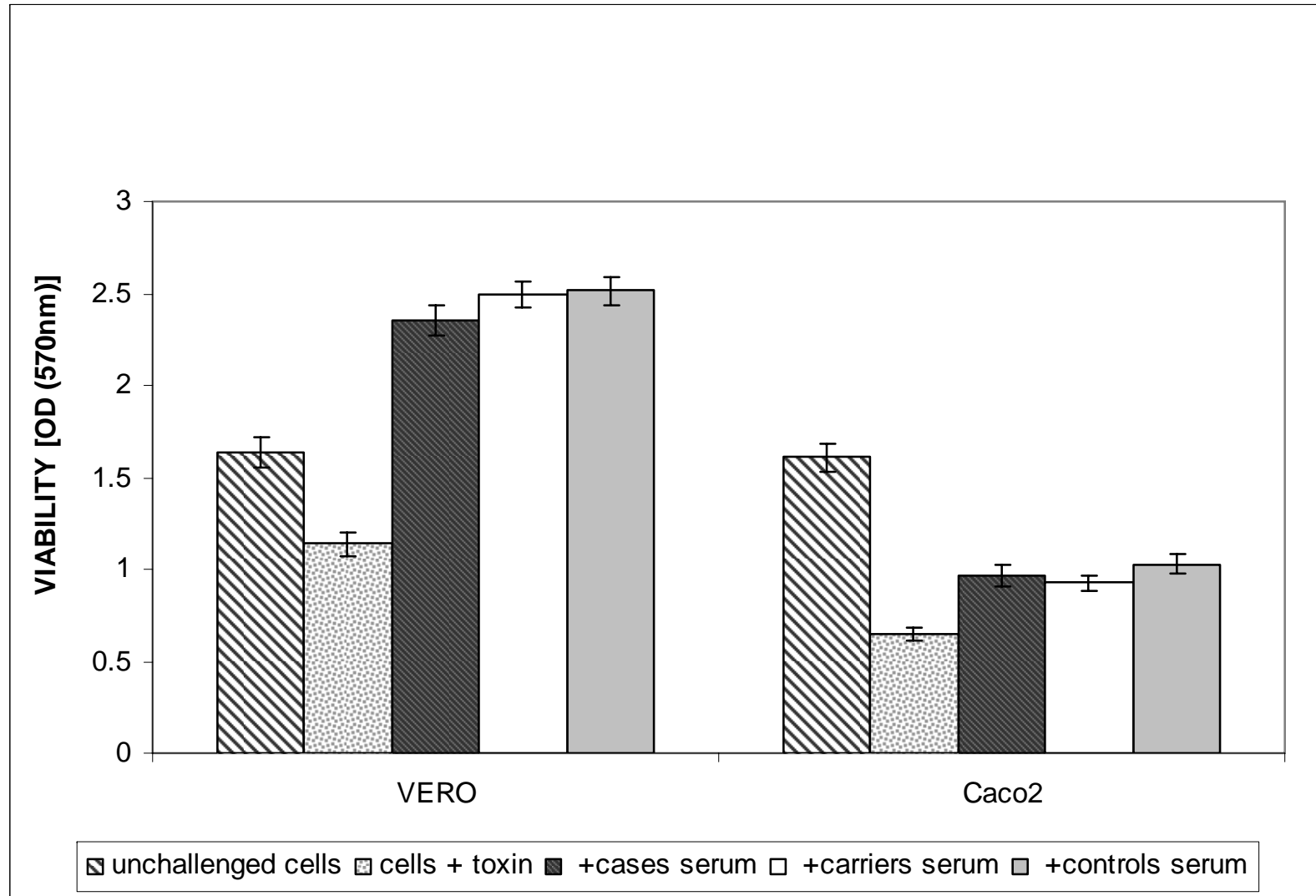
To the following antigens by ELISA:

- Toxin A
- Crude "toxin" - includes all secreted/released bacterial components
- S-layer proteins
- Lipocarbohydrate ("LTA")
- Cell surface complex
- *E. coli* LPS (as a control)

# IgG antibodies - mean levels



# Neutralisation of toxin (MTT assay)



# Systemic antibody response



- "Everyone" has pre-existing antibodies to toxins (neutralising) and cell surface antigens
- Symptomatic patients have highest levels - typical of a "boosting/secondary" response
- No indication of patients being deficient in antibody response - but may have importance in recurrent disease.



# Cellular mucosal responses

Our hypothesis is that comparable immune cell counts will be seen in carriers and controls, and being higher than those seen in CDI patients.

# Cellular responses in colonic mucosa in carriers, cases and controls



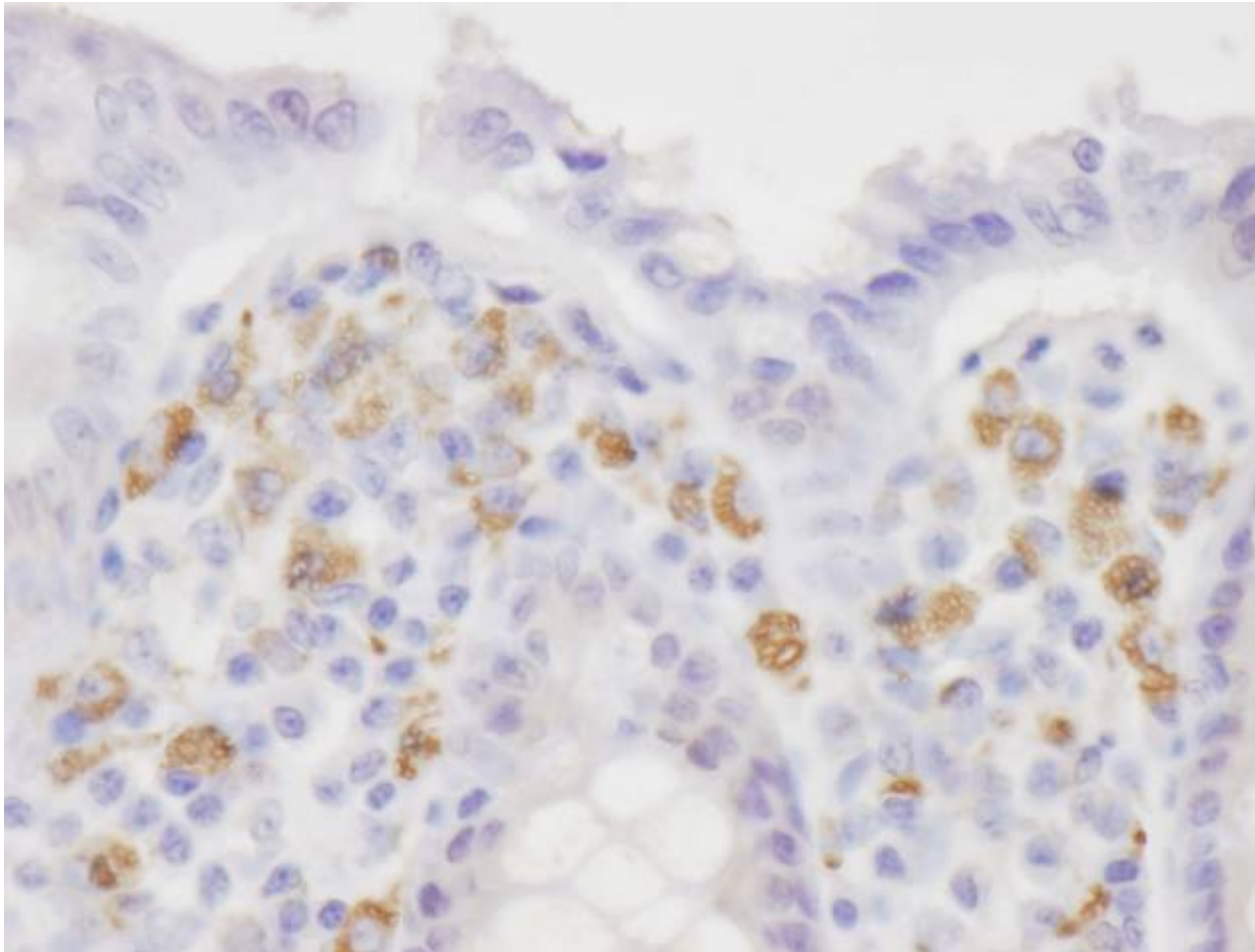
- To date 103 participants from the population of colorectal surgery patients at the Western General Hospital, Edinburgh.
- Stool samples were obtained from all participants and cultured on Brazier's cefoxitin-cycloserine-egg yolk agar
- Three groups:
  - **Cases** - clinically diagnosed CDI (toxin A/B positive stools and/or positive on colonoscopy);
  - **Carriers** - culture-positive (toxin A/B variable) but asymptomatic;
  - **Controls** - culture-negative (toxin A/B negative) and asymptomatic.

# Pathology and immunohistochemistry



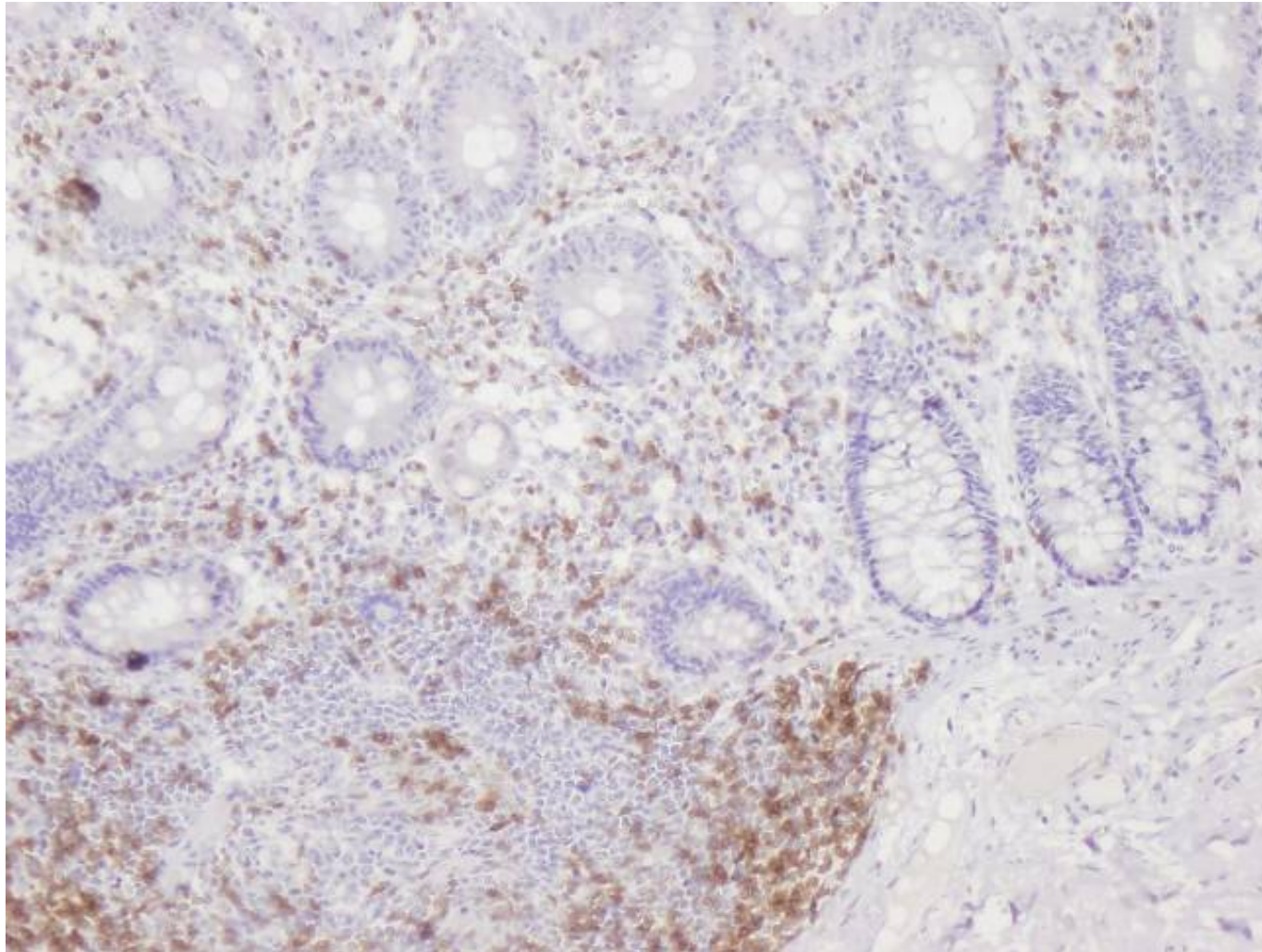
- Normal colonic tissue specimens, taken in the course of diagnosis or treatment by biopsy or resection, fixed in formalin and embedded in paraffin wax.
- Immunohistochemistry was performed using a Vectastain Universal elite ABC peroxidase kit
- The primary antibodies used were specific for
  - T cells (anti-CD3)
  - B cells (anti-CD20),
  - plasma cells (anti-CD138)
  - macrophages (anti-CD68)
- Counts of positively labelled cells in lamina propria by light microscope 10 x 10 grid graticule.
- Ten fields were examined for each slide.

# Macrophages



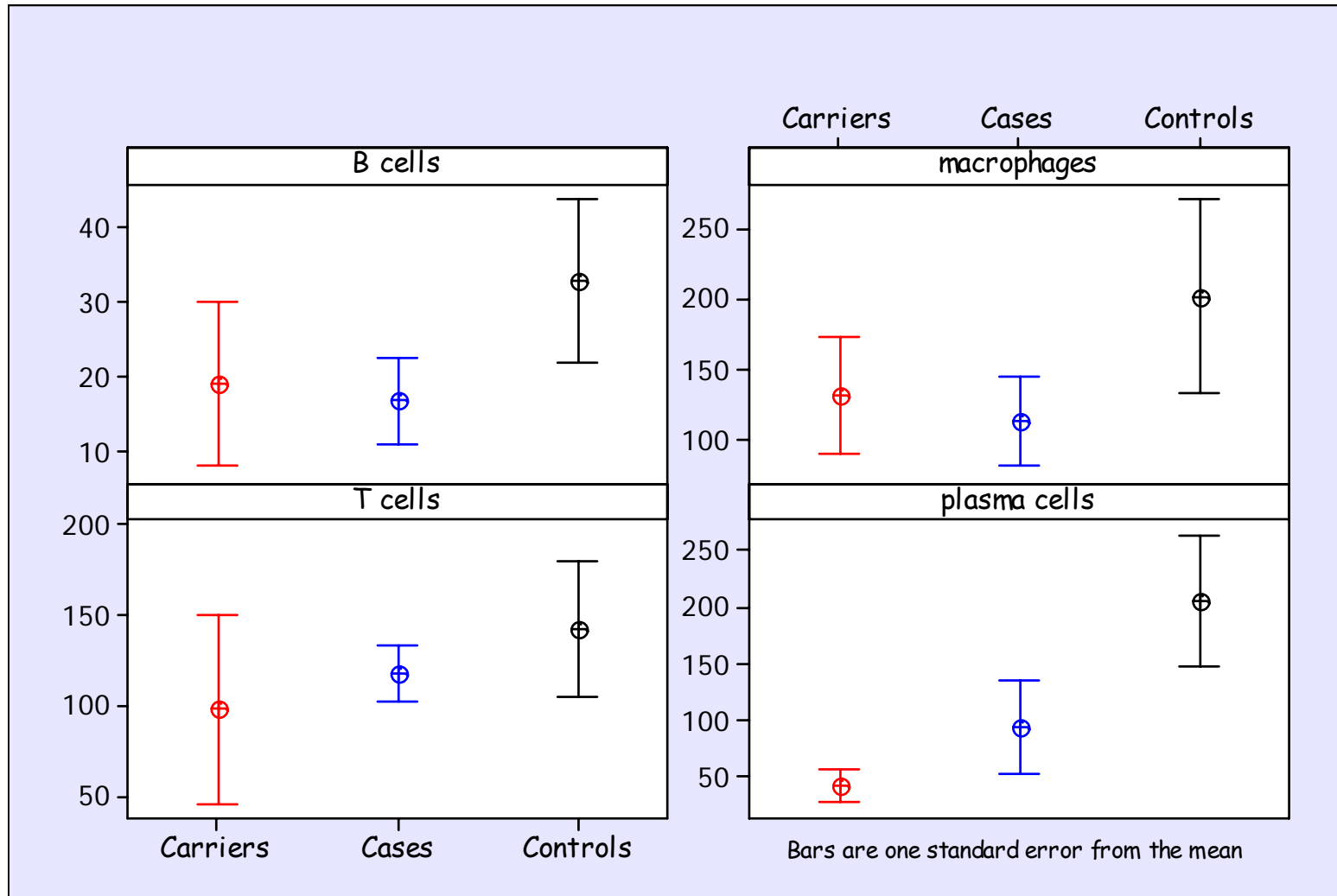
from a  
control,  
CD68  
labelled

# T-cells



from a  
case,  
CD3  
labelled

# Summary of cellular responses in carriers, cases and controls





# Results to date

- Little difference in the mean counts of **T cells** between the three groups ( $p=0.901$ ).
- Mean cell counts of **macrophages** for cases and carriers are lower than controls but not statistically significant ( $p=0.593$ ).
- There is a more marked difference with lower mean counts for cases of **B cells** ( $p=0.415$ ) and **plasma cells** ( $p=0.115$ ).
- But many more slides still left to be counted

# Immunohistochemistry - conclusions to date



- Lower mean B cell, plasma cell and macrophage counts in cases compared with controls agrees with the study of Johal *et al.*, (2004)
- But Johal *et al* only compared cases of CDI with controls
- We also compared carriers and have demonstrated that lower mean counts of these three cell types are apparent for this group also.
- This contradicts our original hypothesis and suggests that features of the host immune response other than reduced cell populations determine a protective response.
- Finally it is very difficult/tedious work

Johal *et al.*, (2004). Colonic IgA producing cells and macrophages are reduced in recurrent and non-recurrent *Clostridium difficile* associated diarrhoea. *Journal of Clinical Pathology*, 57,973-979.

# Conclusions/questions



- Host susceptibility is important in CDI but is very complex to study
- Requires good collaboration with surgeons/other clinicians, pathologists and microbiologists
- Longitudinal studies are required beginning early in course of disease, or ideally before onset of disease.

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