Mycobacterium paratuberculosis and Crohn's disease

This is what Epidemiology is all about

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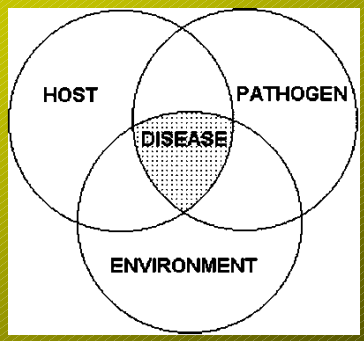


3 main theories for etiology for CD

1) Autoimmune theory
2) Immune deficiency
3) ***Mycobacterium paratuberculosis***

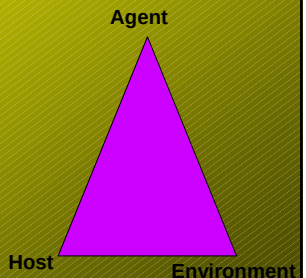
"Rather than competing with one another, the theories may be complimentary "
(Chamberlin & Naser, 2006 ***Med Sci Monit 12;RA27***)

What is epidemiology about?

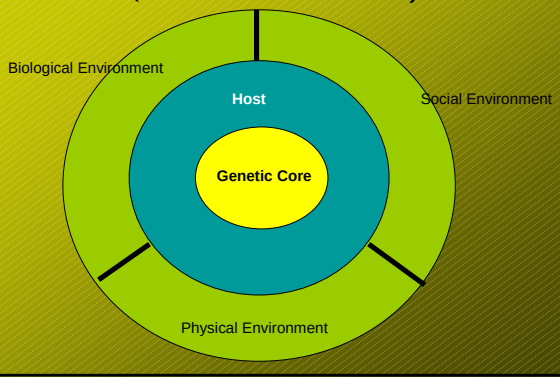
- Study of disease in the population
- A scientific inquiry into the causation of disease
- The search for the risk factors that cause the disease

Epidemiological models for causation

- All components are equally important in disease causation
- Change in any one of them would change the frequency of disease
- Apply to infectious and non-infectious diseases



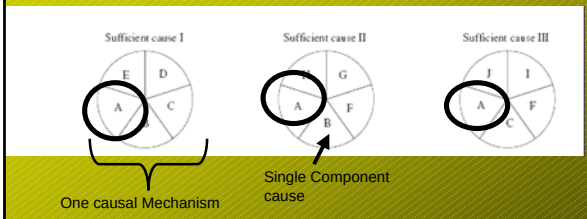
Wheel of Causation (Mausner &Bahn, 1986)



Sufficient & Necessary

- **Sufficient cause:**
  A set of minimal conditions and events that ALWAYS result in a disease.
  Minimal: All of the conditions and events are necessary to that occurrence
- **Necessary Cause**
  A component that must be present but might not be the cause of a disease to develop.

Rothman & Greenland 2005



A is a necessary cause since it appears as a member of each sufficient cause complex

- If the disease does not develop without the factor being present, then the causative factor is termed **NECESSARY**
- If the disease ALWAYS results from the factor, then the causative factor is termed **SUFFICIENT**

Causal Criteria

1) Strength of Association
2) Consistency of effect
3) Specificity of effect
4) Time Order
5) Dose response
6) Biological Plausibility

Measures of Association

A brief reminder .....

Relative Risk (RR)

- The ratio of the incidence of disease in exposed individuals to the incidence in unexposed individuals

Odd Ratio (OR)

- The ratio of the probability of an event occurring to the probability of it not occurring

OR=1 No association
OR>1 Positive Association
OR<1 Negative Association (Protective)

The evidence…
for an association between Map & CD

- PCR identification of Map DNA
- Serum specific Map antibodies
- Imaging of Map DNA by in-situ hybridization
- Culture of Map from tissue, milk & blood

Study Design

- Case-control
- Cohort
- Cross sectional
- Randomized Control Trial
- Challenge Trial
- Prevalence Studies

PCR

- Because of Map's fastidious and slow growing characteristics, its accurate identification is heavily reliant on PCR

Bull et al (2003)
J Clin Microbiol41;2915

Map was identified in:

- 92% (34/37) of CD patients

Vs

- 26% (9/34) in negative controls

	CD+	CD-
PCR+	34	9
PCR-	3	25

OR = 32 (CI 95% 8-128, p<0.01)

Autschbach et al 2005, Germany
Gut 54; 944

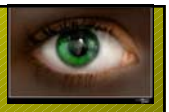
PCR detected the IS900:

- In 52% of 100 CD patients
- 5% of 100 control
- OR= 21

Bernstein et al 2003
J. Clin. Micro. 41;4986

- Case-Control study design
- None of CD patients were PCR positive
- 32% of 19 healthy controls were PCR +

Ojo



- PCR data can be criticized !
- The technique detects DNA from either :

1) Live bacteria

2) Scattered fragments of killed bacteria

Laser Capture micro-dissection

- Map DNA associated with granulomas would not be viewed as scattered debris

Ryan et al (2002) *Gut 52;665*

- 40% of 15 CD cases had Map DNA
- 0% of 12 controls

However…

- Same authors
- E. coli DNA in CD granulomas

Serum antibodies

Naser et al, 1999

Clin Diag Lab Immunol 6; 282

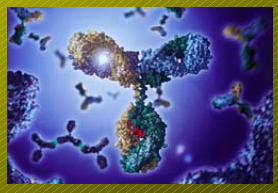
- Presence of IgG :

77% of 61 patients were positive

VS

8% (1) of 12 control

OR=37



Culture evidence

Culture from Intestinal Tissue

Hermon-Taylor et al (2003) *J. Clin. Micrbiol 41;2915*

- 42% (14/33) CD bowel pinch biopsies
  VS
  9% (3/33) controls
- OR=7.4

Milk Culture

- Naser et al (2000)

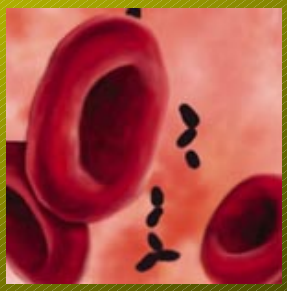
2/2 breast milk samples from lactating CD patients
VS
0 of 5 normal controls

Blood cultures

Naser et al (2004) *The Lancet 364;1094*

50% 14/28 CD patients
VS
0/15 healthy controls

- 2/9 ulcerative colitis also grew Map from their blood



Retail Milk

Abubakar et al, 2007 *Am J Epidemiol. 165;776*

- Consumption of pasteurized milk (kg/month) was associated with a reduced risk of CD
- OR = 0.82, 95%CI: 0.69, 0.97
- Meat intake was associated with a significantly increased risk of CD
- OR = 1.40, 95%CI: 1.17, 1.67

Clark et al 2006 *Mol Cell Probes 20;197*

- A total of 98 retail cheese curd samples were tested for Map by
- PCR
- Culture on Herrold's egg yolk agar
- No viable Map were cultured
- 5% of the samples were PCR positive

Ellingson et al, 2005

J Food Prot. 68; 966

- 702 pints of retail whole milk from CA, MN & WI were tested for the presence of viable MAP.
- Viable Map was detected in 3% of the retail whole milk pints tested.
- Number of samples containing viable Map was similar among states (P > 0.05),
- More positive samples were identified between July through September; Seasonal effect (P = 0.05).

If so ..

- Are people that have frequent contact with dairy cows (dairy farmers..) more susceptible to CD?

Jones et al 2006

Epidemiol Infect.134; 49

- Objective : To determine whether exposure to clinical cases of JD is a risk factor for CD
- Cross sectional study
- Questionnaire to dairy farmers with & w/o reported JD
- NO association between JD and CD was found

It is important to consider…

- Level of exposure is representative
- Controls from an appropriate source population
- Study population representative of the target population and selected randomly
- Intervention randomly assigned

Bigger is Better

- When it comes to sample size !

Scanu et al, 2007

Accepted to JCM

	Map +	Map -
IBS +	15	5
IBS -	3	17

OR =17

"People with Map infection were 17 times more likely to have IBS than people without Map infection"

	IBS +	IBS -
Map+	15	3
Map -	5	17

OR=17

People with IBS are more likely to have Map than people without IBS

What come first ?

Is Map

- An opportunistic bacteria sporadically detected in the general population ?
  i.e. :
- CD patients
- Patients with similar or different diseases
- **Apparently** healthy individuals

Waddell et al (2008)

Can. J. Public Health

- Reviewed 75 relevant papers in English that were suitable for quality assessment
- Sixty Case-Control studies (CCS) were had acceptable quality
- Association also was investigated in 2 challenge trials & 1 cross-sectional study.

Positive association between Map & CD

- Detected in 23 (38%) of 60 CCS
- No association 23 (38%)
- No Map detected in 14 (24%) studies

Case Control Studies

- Large variability among laboratory tests, test protocols, sampling protocols, sample storage and source of population
- Lack of agreement among researchers on how to study this association
- Se & Sp of lab tests was rarely stated (false negative and positive results?)
- Misclassification bias with other IBD

Publication Bias

Conclusions

- Evidence for the association between Map & CD **is not strong!!**
- Zoonotic potential **must not be ignored !**
- Future studies need a **larger sample size !**
- Standardization of sampling
- Appropriate methods for Meta-Analysis

Questions

Thanks