The “Dirt” or Hygiene Hypothesis

Straham Quote: “The rise in childhood allergy is due to improved sanitation, compulsory vaccination, fewer siblings, limited exposure to house dust, pollen and animal dander”

Increase in allergy is associated with affluent countries
Peanut allergy tripled and food allergy increased 18% in ten years in the USA
Increased frequency cannot be explained by changes in the genetic makeup of the countries studied

Studies in humans which support the hypothesis
Allergy and asthma lower in European farm children
Larger families with greater exposure to infectious disease have less allergic rhinitis
Early exposure to peanuts reduce risk of peanut allergy [recently recommended]
Early helminthes (worm) or H. pylori infections associated with less allergy [Elliott lecture]
Farm children raised with animals have less allergy
The Amish- Hutterite experiment
   Asthma and allergy is 4-6 fold lower in Amish children
   Dust from Amish households suppressed airway hypersensitivity when transferred to mice

The hygiene hypothesis is being replaced by the “Microbiome Hypothesis”
1. 14 of 17 studies supporting the hygiene hypothesis show a link between the gut microbiota and atopy
2. Allergic children have a lower microbiome diversity before they develop allergy
3. Allergic children have higher levels of Enterococcus, Clostridia and lower levels of Lactobacilli
The Increase in Childhood Allergy is Paralleled by Increases in other Maladies and Practices in Western Societies in the Last 60 Years

Changing Practices
- Increase in Caesarian deliveries
- Shift to high protein and carbohydrate diets
- Use of antibiotics
- Decrease in breast-feeding or duration of breast-feeding
- Use of antibiotic growth promoters (AGP) in food animal production
- Urbanization and transcontinental air travel

The Increase in Disease temporally parallels these changing practices and in the rate of antibiotic prescriptions

[Obesity, IBD, Autism, Gastrointestinal reflux disease (GERD) childhood & adolescent allergy and asthma]
Studies Implicate Antibiotics, an Unhealthy Microbiome and the failure of Tregs

Mouse studies correlate IBD and food allergy with an unhealthy microbiome:
- GF mice cannot develop oral tolerance until colonized with Clostridium
- Non-tolerant mice have very few Tregs
- Oral tolerance can be transferred to GF mice with the microbiome from conventionally-reared mice
- Tolerance is associated with production of SCFA that appear necessary for Treg development and healthy tight junctions (Prevotella/MS study)
- Mice lacking the TLR receptor for endotoxin/ LPS are highly susceptible to food allergy

Antibiotics contribute since mice given a broad-spectrum antibiotic to reduce/eliminate their microbiome:
- 1. Develop elevated levels of food allergy compared to controls
- 2. Show poor development of Tregs
- 3. Develop airway inflammation and hyperactive airways when aerosol challenged with house dust

The microbiome of allergic children shows:
- 1. An over-representation of Enterobacteria (Proteobacteria) and a lower proportion of Bacteriodetes
- 2. The feces of allergic children contain lower levels of SCFA produced by Bacteriodes
- 3. Resolution of food allergy is associated with an enrichment of Firmicutes, e.g. Clostridium
Antibiotic Growth Promoters (AGP)

History and Use

1940s: Serendipitous discovery that feeding the waste from the pharmaceutical production of tetracycline to chickens resulted in a significant weight gain [The waste contained aureomycin!]

1946-1950: Unambiguous evidence that Sub-Therapeutic Antibiotic Treatment (STAT; mostly penicillin) resulted in a 15-20% weight gain in chickens and swine (p<0.001). Approved for use in UK and EU

1960-1990: Period during when using STAT, now called AGP, became standard practice in agriculture.

1999: EU banned use of avoparcin and four additional antibiotics for use as AGP

2000: WHO recommended that antibiotics used in human medicine not be used as AGP

2002: McDonalds and KFC stopped using chicken meat raised with AGP

2003: First mention by FDA of the dangers associated with use of medical antibiotics in food animals

2007: US banned fluoroquinolones for use in food animals but not in pets

2012: AVMA Fact Sheet for veterinarians. Medical antibiotics, including Tet, can only be used therapeutically but can be and administered to all animals in a barn regardless of their health !!!

AGP is a major industry

1. 300 million Lbs./yr. or 200 g/ton of animal feed which translates to 320 mg AGP to produce 1 kg (2 lbs.) of meat

2. 80% of all antibiotics produced are used as AGP

3. Prior to 2002, 200,000 Lbs. (mostly tetracycline) was used per year in Denmark alone

4. 90% of pig starters, 70% of “grower diets” and 50% of “finisher diets” contain STAT/AGP
AGP Promotes Weight gain and Requires the Microbiome

1. A 25 yr. study in piglets demonstrated a 16% average gain in weight and 7% increase in feed efficiency

2. Typical study in piglets which measures the results in feed costs per weight gain, i.e. lower numbers are better.

AGP Mixture Used for Feeding

<table>
<thead>
<tr>
<th>Growth Period</th>
<th>Control</th>
<th>F</th>
<th>FOA</th>
<th>CSP</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-37 days</td>
<td>2.76</td>
<td>2.54</td>
<td>2.43</td>
<td>2.6</td>
</tr>
<tr>
<td>37-121</td>
<td>3.44</td>
<td>3.54</td>
<td>3.64</td>
<td>3.44</td>
</tr>
</tbody>
</table>

3. Studies in poultry (broilers) from 1950-200 showed a 15-20% average increase in wt. when reared using AGP

4. Weight gain requires a microbiome
   1. Piglets and mice reared GF weigh only 60% of their colonized littermates reared on the same diet
   2. Conventional mice given a high fat diet gain more weight than controls but requires STAT to double their weight
   3. The weigh gain advantage in STAT-treated mice can be transferred to GF mice with the GIT microbiome

5. Antibiotics, AGP and “Original Sin”
   1. STAT/AGP must be given early to have any effect on weight gain (see above)
   2. Early use of antibiotics in pre-terms has long-lasting effects on the composition of the microbiome
   3. STAT-induced alterations in the microbiome of mice persist for life
The Consequences of the Withdrawal of AGP

Negative consequences
  An increased mortality of piglets to necrotic enteritis that increased from 2.7 to 3.5% after withdrawal
  A decrease in feed efficiency in piglets reared in Denmark
  A greater chance for development and spread of resistant pathogens (speculation!)
  An increase in pathogen levels in dairy cattle when AGP was discontinued in Europe
  A tendency to resort to greater use of therapeutic antibiotics, e.g. Tetracycline [Denmark and Holland]
  Loss of revenue for Big Pharma!!
  Increase in the number of sows to compensate for the decrease in weight gain and the increased piglet mortality

Positive consequences
  Danish Poultry Council reported that withdrawal did not decrease weight gain
  Perdue farms say that not using AGP actually saved one cent per chicken because of the costs of AGP!
  Recovery of AR *Enterococcus faecium* dropped from 80% to 5% in Danish pigs after the 2000 ban on GP
  Reduced “antibiotic pressure” reduces selection for AR genes (see later)
  Provides a stimulus to identify alternative methods such as the use of pre- and probiotics

Not surprisingly, we only have speculation as to how AGP increases animal production
  1. Reduces microbiome size allowing more nutrients to be directed to the host! [Isn’t low diversity unhealthy?]
  2. Preferentially reduces pathobionts levels to allow expansion of Bacteriodetes that convert fiber to SCFA
  3. Reduces pathogen pressure on the host immune system thus providing more energy for growth
  4. Reduces need for building a “defensive mucosa: allowing greater nutrient absorption! [Less protection is good?]
Can the Effect of AGP and Antibiotics be Extrapolated to Humans?

The obesity epidemic

Obesity steadily increased since 1960, doubled in the last two decades and is highest in the USA (36%)
Obesity is linked to “Western” diets high in red meat and low in fiber
400 million adults are obese and it is linked to Type II diabetes, higher levels of TMA and CVD
In 2017, more Americans died from obesity than from smoking
42 million children under the age of 5 were overweight in 2013

Is obesity associated with changes in the microbiome?

Overweight European children have more Enterobacteria (pathobionts) and less Bacteriodetes and Firmicutes
Is the Firmicutes: Bacteriodete ratio is a marker of obesity?
In a study of twins, obesity is associated with lower diversity of their microbiome
Diet may allow expansion of the GIT microbiome that craves eating more junk food;
i.e. activation of the gut-brain axis. (see Gundry on the Internet)!

Do antibiotics and AGP cause obesity?

Weaned mouse pups given STAT are fatter at 16-24 weeks than controls with a 30-60% increase in body fat
Children who received antibiotics in the first 6 months of life (but not after) are fatter than controls
If a microbiome is needed for weight gain and antibiotics destroys the microbiome, why obesity?

Interesting read (?): Steven Gundry: “Dr. Gundry’s Diet Evolution; Turn off the genes that are killing you”
Horizontal Transfer of Pathogens from Animals to Humans

Horizontal transfer is a well-known threat to human health. Emerging viral diseases have held the recent limelight:

- HIV = chimpanzee
- Influenza (H1N1) = swine
- Ebola = fruit bats
- West Nile = horses
- SARS = Chinese horseshoe bat
- Rabies = carnivores
- Zika = Aedes mosquito?

Transfer of antibiotic-resistance bacteria or antibiotic resistance genes (AR Genes) are a major health threat:

“Two million Americans annually get infectious diseases from bacteria with AR Genes and 23,000 die”. Tom Frieden (CDC).

The annual cost to health care of AR Genes in the USA is estimated at 21-34 billion dollars

Evidence of horizontal transfer of AR Genes or pathogens:

- 1976: Transfer of tetracycline-resistant plasmid by E. coli in chickens
- 1997: Vancomycin-resistant Enterococcus transferred from turkeys to turkey farmers
- 2000: Avoparcin-AGP (glycopeptide) resistant Enterococcus transferred from swine and poultry to farm workers
- 2002: Fluoroquinolone resistant S. enterica transferred from swine to hospital patients in Korea
- 2003: Level of tetracycline-resistant soil bacteria elevated in soil sprayed with hog farm manure
- 2006: Major source of S. enterica (choleraesuis) in Taiwan hospitals is from pigs that developed resistance to fluoroquinolines but 60-80% are also resistant to chloramphenicol and ampicillin (co-selection)
- 2008: Meat and pigs imported to Denmark contain methicillin-resistant Staph aureus (MRSA)
- 2013: Livestock origin of pandemic MRSA clone demonstrated
- 2016: Plasmid-borne colistin resistant gene (mcr-1) in Enterobacteria of four continents has an animal origin
The GIT Microbiome as a Reservoir of Antibiotic Resistance (AR) Genes

The microbiome normally contains AR Genes or antibiotic-resistant pathogens

1. AR Genes can be transferred by the normal mechanisms of bacterial mating (“Bacterial Porn”; left below)

2. AR Genes and others responsible for pathogenesis can be maintained in non-pathogenic mutualists within the healthy microbiome and transferred horizontally among phyla to pathobionts

3. AR Genes are inadvertently co-selected (see later)

The encircled genes can be transferred among microbial phyla of the microbiome

- **ermB** confers resistance to erythromycin which is responsible for pathogenesis in *Salmonella typhimurium* and *Clostridium perfringens*

- **Tet** and **TetQ** are tetracycline resistance genes

Transfer is often by plasmids
AR Gene Expression can Occur by Co-selection

**Summary:** Three different mechanisms that can result in AR gene expression

1. Therapeutic antibiotic or AGP exposure selects for spontaneous resistance to antibiotics
2. Environmental antibiotics, e.g. from Streptomyces, and other soil bacteria may select for AR expression
3. AR genes may be co-selected as part of a “DNA island” that is under control of a common promoter

Bacterial genes occur in clusters preceded by a common promoter sequence (P)

![Diagram of bacterial gene clusters with promoters (P) and repressors (R) with RNA polymerase initiating transcription. Multigene clusters are indicated.](image.png)

RNA polymerase attaches to the promoter (P) initiating transcription of all the genes in the downstream cluster provided that this process is not stopped by the repressor (R).

Proteins and metabolites, e.g. lactose, can attach to the repressor to **switch it off** allowing transcription of all genes in the multigene cluster to proceed.

For example, a high concentration of lactose in the diet can **switch off** the repressor to allow the enzyme lactase to be expressed and to cleave lactose into glucose and galactose (simple sugars).

The particular cluster may contain a genes needed for a particular enzyme, e.g. lactase (E) but also an AR genes (AR)

![Diagram of lactase (E) and AR genes within a multigene cluster.](image.png)

A metabolite like lactose could then switch off the repressor resulting in the expression of the gene for the enzyme lactase (E) and simultaneously the gene for antibiotic resistance (AR).
Examples of Co-selection of AR genes

Enterococcus resistance (GRE) to the glycopeptide avoparcin (a AGP) was studied in Danish swine herds

1. Since avoparcin use was prohibited, there was a decline from 80-5% in GRE in Denmark and to a lesser extent in Germany and Italy of Enterococcal infection in swine herds

2. However, GRE resistant pigs were also resistant to the macrolide erythromycin

3. AR genes for GRE (vanA) and erythromycin (ermB) are both closely located on a transmissible plasmid.

Nosocomial outbreaks due to antibiotic resistant Enterobacteria (e.g. E. coli) to aminoglycosides have increased

1. These E. coli strains are also resistant to gentamicin, kanamycin, sulfonamides and cephalosporin

2. Plasmids from gram-negative Enterobacteria (e.g. E. coli) also contain AR genes for cephalosporin and other aminoglycosides.
Vectors in the Transmission of AR Genes

Transmission increases with Proximity

“Farmer’s Lung” was first identified in farmers in the mountains of Switzerland who lived in the same house as their cattle.

“Pigeon Breeder’s Disease” was first found in fanciers of pigeons who raised them in lofts in their homes.

Vancomycin-resistant *Enterococcus* in turkeys was identified in turkey farmers and MRSA was first recognized in swine farmers in France and Holland.

Livestock have long been a reservoir of AR genes [see Appendix]

Summary of the Pathways taken by AR Genes

- The GIT harbors AR Genes available for transfer during bacterial mating
- Farm workers inhale air in confinement barns containing AR bacteria
- Contamination by feces can allow transfer *i.e.* from lagoons or manure spreading

Insects as vectors

- 1999: Houseflies in Japan are reservoirs for *E. coli* 057:H7 [Jack-in-the Box pathogen]
- 2006: 97% of houseflies in NE Kansas harbor Enterococcus resistance to kanamycin, tetracycline and ciprofloxacin
- 2008: Within 30 minutes, five houseflies deposited $10^3$ CFU of antibiotic-resistant enterococcus on a hamburger
- 2009-2012: Gypsy moths and honey bees harbor tetracycline and oxytetracycline resistant bacteria (used for blight)
- 2013-2014: Cockroaches in a food-handling facility and hospital carry antibiotic-resistant pathogens
Facts, Fiction, Fake News and Misunderstandings about AGP and Therapeutic Antibiotics

There is **no evidence** that antibiotics/AGP used in food animals are transferred in meat. “Vegan Fake News”

**Vegans beware!** Oxytetracyline and streptomycin used to treat apple and pears blight are transferred

Prior to modern regulations, 50 micrograms tetracycline could be ingested by drinking two cups of milk. Tetracycline is no longer allowed to treat animals

**More Vegan fake news:** Hormone transfer is insignificant regardless of warnings

Antibiotics **do not create** antibiotic-resistance genes (AR Genes)

AR Genes results from random genomic mutations at the rate of $10^{-9}$ per bacteria per generation

Removal of antibiotics does not remove AR Genes or decrease the rate of mutation

Most AR Genes persist in non-pathogens where they “hide” and do little harm

Antibiotics pressure selects for proliferation of those bacteria that carry AR Genes

**1976 Experiment:** Tetracycline (Tet) -resistant plasmid ($pSL\ 222-6$) introduced into $E.\ coli$ and used to inoculate chickens

<table>
<thead>
<tr>
<th>Inoculant</th>
<th>Feeding regime</th>
<th>Chickens with plasmid after 50 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>$p\ SL\ 222-6$</td>
<td>Tet AGP (Pfizer)</td>
<td>44</td>
</tr>
<tr>
<td>$p\ SL\ 222-6$</td>
<td>Control feed</td>
<td>0</td>
</tr>
</tbody>
</table>

Other examples

1. Increase frequency of antibiotic resistant $S.\ pneumoniae$ parallels long-term penicillin usage
2. Highest rate of cephalosporin-resistant bacteria parallels highest usage in southern Europe (Italy)
3. Highest incidence of antibiotic-resistant $S.\ typhimurium$ (DT104) followed introduction of fluoroquinolone AGP
Replacing AGP with Pre- and Probiotics in Agriculture and Aquaculture

History

1906: Tissier showed that Bifidobacteria could displace pathogenic bacteria from the GIT

1940: *S. aureofaciens* could facilitate weight gain in animals because it produced its own chlortetracycline
The Effect of Probiotics on Weight Gain in Animal Production

A. *Lactobacillus sp* increases weight gain in broiler chicks and ducks

<table>
<thead>
<tr>
<th>Species</th>
<th>Day</th>
<th>Weight</th>
<th>Day</th>
<th>Weight</th>
<th>p  value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broiler chicks</td>
<td>0</td>
<td>94.2</td>
<td>0</td>
<td>88.5</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>1623.1</td>
<td>60</td>
<td>1878.3</td>
<td><strong>0.0064</strong></td>
</tr>
<tr>
<td>Ducks</td>
<td>0</td>
<td>82.8</td>
<td>0</td>
<td>85.2</td>
<td>0.86</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>2472</td>
<td>60</td>
<td>2876</td>
<td><em>0.035</em></td>
</tr>
</tbody>
</table>

B. Weight gain associated with increase in Firmicutes and Firmicutes:Bacteriodetes ratio (see below)

Black = control   Red = One dose   Green = Two doses
The Efficacy of Probiotic Therapy

Trials using probiotics to reduce allergy during childhood (not in pre-terms) are ambiguous. 17 trials (2947 infants) indicated that post-natal treatment reduced the risk of sensitization to food allergens. European Academy and World Allergy Organization concluded that except for eczema, evidence is too weak to recommend usage. **[Refer to remarks by Dr. Ziegler]**

Strongest support for probiotics comes from:
- Use of pre- and probiotics in pre-term and Caesarian-derived infants
- Restoration of the microbiome after long-term antibiotic therapy

Questions abound concerning the use of probiotics in healthy individuals to maintain good health:
1. The recommended daily dosage of probiotic bacterial yogurt of $10^8$ to $10^{10}$ would add one probiotic bacteria to **10,000 to one million** bacteria in the GIT biome, **“A piss in the ocean”**
2. If an increase in the Firmicutes:Bacteriodete ratio is a marker for obesity, why use Lactobacillus (a Firmicutes) as a probiotic?
3. If Bacteriodes are critical, [major sources of healthy SCFA], how do you deliver a strict anaerobes in a probiotic food or drink? Is fecal transplant the only way?
4. Maybe a high fiber diet, that encourages Bacteriodetes is the wiser choice

The efficacy of probiotic as an alternative to AGP in food animals
Using probiotics would obviously raise the cost of production compared to AGP
Variations in the time of administration has been very poorly studied
Falls into the same category as the argument for “Green Energy” versus petroleum.
“Eat Your Yogurt” and “Do no Harm”: A Summary

More should be done to limit use of antibiotics, the “20th century miracle drug”:
- In diseases that are clearly of viral origin, 30-50% of prescriptions should not be written!
- When antibiotics are given long-term to patients with infectious diarrhea like *C. difficile*
- When the antibiotic sensitivity of the infecting bacteria is unknown
- In patients with certain types of prostheses and in certain dental procedures

Pre- and probiotics, the “21st century wonder drug” can be valuable for:
- C-section pre-term infants that did not obtain a microbiome through conventional birth and/or suckling
- Patients recovering from long-term antibiotic therapy and suffering from diarrhea
- Providing a placebo effect for users who think it makes them healthier, the “Eat Your Yogurt” group

AGP are a valuable tool in animal production but:
- Do not produce a higher rate of genomic mutation leading to a greater number of AR Genes
- Are not transmitted to the human consumer with possible exception of cows milk and raw eggs
- Encourage the expansion of bacteria with AR Genes and spread to farm workers

The promotion of pre- and probiotics lags way behind the science that can firmly support their use, however:
*Folklore has often preceded science and has often been proven correct*
- Compared to pharmaceuticals, there is no evidence that “Eating Your yogurt” does any harm and yogurt alone is a tasty and healthy product.
Diet, Culture, Ethnicity and the Microbiome: Diagrammatic Summary

Appendix
Livestock have long been a reservoir for AR Genes

MRSA has been one of the best studied examples
MRSA ST130 strain spread from cows to humans
MRSA CC97, a major cause of mastitis, spread to humans in Denmark and increased 11-fold in five years
Transfers occur in both directions
Transfers can be traced back many years using modern genetic technology
S. aureus CC59, an endemic in Taiwan, may have originated in livestock 500 years ago
Recent study traces endemic MRSA CC97 back ~100 years

Blue= bovine
Green = porcine
Orange = caprine
Red = human