National Wildlife Rehabilitation Conference Perth, Western Australia 2007

Understanding and managing the gut ecology of Australian wildlife by light-microscopy diagnosis of faeces

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I. Background



Microscopy State-of-the-Art, 1665





Robert Hooke (England)

- first compound microscope
- first observed 'cells' of cork



Microscopy State-of-the-Art, 1673



1683

Anton van Leeuwenhoek (Holland) - 300x single lens; first to describe living 'animalcules' which we now know as bacteria and protozoa



Microscopy State-of-the-Art, 1869



1st compound microscope with modern optics similar to microscopes today, built for Joseph Jackson Lister, the father of the famous surgeon, Joseph Lister



Joseph Lister (1867) Use of Disinfectants in Surgery





Brain Surgery 1900

British Surgical Team 1927



19th Century Discoveries

DATE	DISEASE	ORGANISM	SCIENTIST
1876	Anthrax	Bacillus anthracis	Koch
1878	Infection	Staphylococcus	Koch
1879	Leprosy	Mycobacterium leprae	Hansen
1880	Gonorrhoea	Neisseria gonorrhoeae	Nelsser
1880	Typhoid	Salmonella typhii	Eberth
1880	Pneumonia	Pneumococcus	Pasteur, Sternberg
1882	Tuberculosis	Mycobacterium tuberculosis	Koch
1883	Cholera	Vibrio cholerae	Koch
1883	Diphtheria	Convnebacterium diphtheria	Klebs
1885	Cystitis	Escherichia coli	Escherich
1885	Tetanus	Clostridium tetani	Nicolaier
1886	Pneumonia	Streptococcus pneumoniae	Fraenkel
1887	Meningitis	Neisseria meningitidis	Weichsellbaum
1887	Brucellosis	Brucalla melitensis	Bruce
1889	Tetanus	Clostridium tetani	Kitasato
1892	Gangrene	Clostridium perfringens	Welch, Nutall
1894	Plague	Yersinia pestis	Roux, Kitasato
1897	Botulism	Clostridium botulinum	van Ermengem
1898	Dysentery	Shigeila dysenteriae	Shiga
1905	Syphilis	Treponema pallidum	Schaudinn, Hofmann
1906	Whooping cough	Bordetella pertussis	Bordet, Gengou

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Microbial Sources of Penicillin and Other Antibiotics (1928-Present)



FIGURE 20.1 The discovery of penicillin. Alexander Fleming took this photograph in 1928. The colony of *Penicillium* mold accidentally contaminated the plate and is inhibiting nearby bacterial growth.

New antibiotic-producing organisms are discovered by looking for areas of inhibition around colonies.

table 20.1	Representative Sources of Antibiotics				
Microorganism	1	Antibiotic			
Gram-Positive Bacillus subtilis	Rods	Bacitracin			
Bacillus polymyxa	p.	Polymyxin			
Actinomycetes Streptomyces nod	losus	Amphotericin B			
Streptomyces ven	ezuelae	Chloramphenicol			
Streptomyces aur	eofaciens	Chlortetracycline and tetracycline			
Streptomyces ery	thraeus	Erythromycin			
Streptomyces frac	diae	Neomycin			
Streptomyces gris	eus	Streptomycin			
Micromonospora	purpurea	Gentamicin			
Fungi Cephalosporium	spp.	Cephalothin			
Penicillium grised	ofulvum	Griseofulvin			
Penicillium notat	tum	Penicillin			

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Microphobia 2007

"Don't touch that..."



Beneficial Role of Gut Microbes

- aid in digestion
- neutralise toxins
- food for other microbes
- compete with harmful microbes
- create environment for beneficial microbes



II. Kanyana Microscopy Station

Ruth Haight, RVN

Gerry Waneck, PhD



Former Kanyana Microscopy Station



Former Microscope & USB Camera







Present Kanyana Microscopy Station



Kanyana Microscopy Station Goals

Diagnostics

Interface with hospital: relate findings to clinical signs, determine treatment, follow outcome

Documentation

Record observations: describe poop, identify microbes, record by photo & video, enter into database

Teaching

Teach courses for wildlife carers and vet students, produce training videos, conduct public education

Research

Interface with universities & government agencies, watch for unknowns (new discoveries)



KanyanaKare Database Home Page

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Created by Greg and Sue Hambleton

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KanyanaKare Database Patient Details

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What Are We Looking For?

Abnormalties (What is normal?)

Most knowledge comes from human or veterinary studies Species-specific poop patterns (diverse wildlife species) Food eaten in wild vs captivity (food debris artifacts) Time of year (available food to eat, pollen artifacts)

Signs of Large Parasites

Arthropods (eg, mites): off body, not living in gut Worms: roundworms, flatworms (usually only see their eggs)

Small Parasites

Protozoans (single-celled animals): amebae & flagellates

Bacteria and Yeast Infections
 Population imbalance (eg, monoculture)
 Strange microbes in sick animals



Spotting the 'Bad Guys'

Learn How They Look

Study textbooks and information on the Web Look at a lot of samples (experience) Relative size is an important clue

Learn to Ignore Irrelevant Objects
 Not everything can be identified
 Typical artifacts are food debris, urates, pollen

Be Curious and Suspicious Learn which "irrelevant" objects to ignore Learn case history and clinical condition of the animal

Look Long and Hard
 Some objects are rare
 Some microbes are hard to spot







Worm samples. Top right: Tapeworm. Top left: Roundworm. Bottom: Gizzard worm. Parasite eggs will vary according to the size of the parasite.



Relative Sizes of Parasites (1)



Relative Sizes of Parasites (2)



Relative Sizes of Bird Parasites





Bacteria Types



Gram Positive & Negative Bacteria

Urate Crystals

Microscopic Structure of Plants

Pollen Artifacts ?

'Bad Guys' We Usually See

Worm Eggs

Nematodes: threadworms, roundworms, gizzardworms, pinworms Cestodes: tapeworms Trematodes: flukes

Protozoa

Amoeba: *Coccidia* Motile ciliates and flagellates: *Trichomonas*

Yeast

<u>Avian Gastric Yeast of parrots: Macrorhabdus</u> (Megabacteria) Budding and germinating yeast: *Candida*

Bacteria

Monocultures: liquid phase suspension; cocci biofilms 'Strangers': unusual flora for species; spore-forming rods

Worm Eggs: Threadworm

Worm Eggs: Roundworm

Worm Eggs: Gizzard Worm

Worm Eggs: Pinworm

Worm Eggs: Tapeworm

Worm Eggs: Flukes

Mature

Immature

Protozoa: Coccidia

Yeast: Avian Gastric (AGY)

Budding Yeast: Candida

Bacteria: Diplococci Monoculture

Bacteria: Common Motile Rods

QuickTime[™] and a H.264 decompressor are needed to see this picture.

Bacteria: Large Motile Rods

QuickTime[™] and a H.264 decompressor are needed to see this picture.

Bacteria: Motile Rods & Protozoa

QuickTime[™] and a decompressor are needed to see this picture.

Bovine Rumen Microbes

QuickTime[™] and a H.264 decompressor are needed to see this picture.

III. Case History: Gut Infection in a Western Gray Kangaroo Joey

Polyester Pouch Fibres

Large Spindle Rods

Clostridium difficile (Scanning EM)

White Blood Cells in Faeces

"Pseudomembranes" in Faeces

Pseudomembranous Colitis

Lanne Friesen and Nicholas Woothdge

C. difficile vegetative cells produce toxins A and B and hydrolytic enzymes (1). Local production of toxins A and B leads to production of tumour necrosis factor-alpha and proinflammatory interleukins, increased vascular permeability, neutrophil and monocyte recruitment (2), opening of epithelial cell junctions (3) and epithelial cell apoptosis (4). Local production of hydrolytic enzymes leads to connective tissue degradation, leading to colitis, pseudomembrane formation (5) and watery diarrhea.

Fig. 1: Pathogenesis of Clostridium difficile-associated diarrhea in adults.

Clostridium difficile (Gram stain)

Western Gray Joey: Intestine Ulcerative Inflammation (1)

Western Gray Joey: Intestine Ulcerative Inflammation (2)

Western Gray Joey: Liver Haemorrhagic Necrosis

Potential C. difficile Epidemic

Epidemic Clostridium difficile

Thomas V Riley

MJA • Volume 185 Number 3 • 7 August 2006

We need to know if and when this organism arrives in Australia

"Natural" therapy for infectious diseases

Clayton L Golledge and Thomas V Riley

Introduce the right kind of bacteria and several pathogens lose their niche in the human host -- that's the rationale behind probiotic therapy. These and some other "natural" therapies are popular with patients and increasingly supported by research results.

MJA 1996; 164: 94

Aussie Full-Cream Pot-Set ABC Yoghurt

Margaret River & Mundella Ingredients

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Retorology	000000	genco	✓ No preservatives
Calcium	171mg	171mg	1 Calcium rich
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