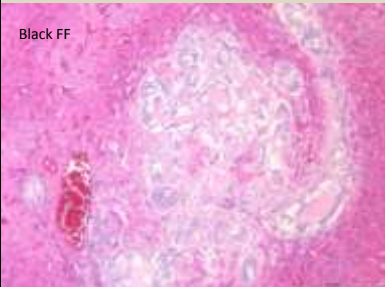


Slide 1

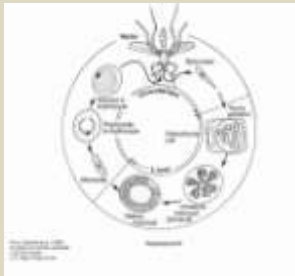


***Hepaticystis* spp. in bats**

- also occur in primates, rodents, ungulates
- *Culicoides* spp vector
- formation of “merocysts” in liver, which fill with colloid



Black FF



- mostly no associated pathology described
 - very rare fatalities in primates
- sporadic finding in FF livers at BSL
 - almost always incidental

Slide credit: Anita Gordon

Hepaticystis is a haemosporidean parasite which is well described in bats and also occurs in a variety of other mammals.

Life cycle involves a *Culicoides* vector injecting a sporozoite which goes on to infect a hepatocyte and form large merocysts with central eosinophilic colloid

Biosecurity sciences laboratory

Background: Haemosporidian parasites in bats

Haemosporidian genera reported from mammals		
Genus	Host(s)	Vector
<i>Plasmodium</i>	primates, bats, rodents	<i>Anopheles</i> spp
<i>Hepatocystis</i>	primates, bats, rodents, ungulates	<i>Culicoides</i> spp
<i>Rayella</i>	rodents	unknown
<i>Nycteria</i>	bats	unknown
<i>Polychromophilus</i>	bats	Nycteribiidae (bat flies)
<i>Johnspretia</i>	bats	unknown
<i>Sprattiella</i>	bats	unknown
<i>Dionisia</i>	bats	unknown
<i>Bioccala</i>	bats	unknown
<i>Biguetiella</i>	bats	unknown

Modified from Schaer et al (2015) <http://dx.doi.org/10.1016/j.ijpara.2015.01.008>

- *Plasmodium* spp dominate the literature
- Seven genera described exclusively from bats, but 5/7 only have a single description

Slide credit: Anita Gordon

Slide 4

- Six adult male LRFF received
March-May 2014
- All from Brisbane/Sunshine Coast
- Neurological signs in bats 1-4, 6
- Bat 5: Trauma
 - Observed to fly into a fence and subsequently attacked by dog
- Respiratory signs in bat 1



Slide credit: Anita Gordon

Pulmonary pathology

- Gross: Lungs mottled, dark red or wet in 4/6
 - difficult to distinguish from XS barbiturate artefact
- Histo: Multifocal granulomatous, eosinophilic pneumonia
 - macrophages, multinucleate giant cells, eosinophils
 - variably angiocentric; sometimes associated with vasculitis
 - associated with round to fusiform to sinuous protozoal schizonts
 - mild (3/6) to moderate (2/6) to severe (1/6)

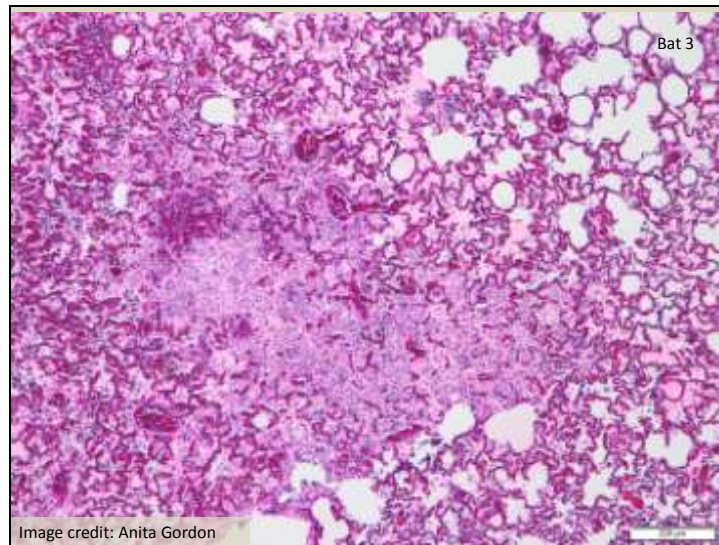
Slide credit: Anita Gordon

Other diagnoses in bats with haemosporidial pneumonia

Bat	Neurological signs	Respiratory signs	Severity of pneumonia	Other Diagnoses
1	+ abnormal vocalisation, paraplegia	+ dyspnoea, abdominal breathing, gurgles in chest	moderate	-
2	+ moribund, poorly responsive, bilateral mydriasis	-	mild-moderate	Head trauma
3	+ on ground unable to move, aggressive	-	moderate-severe	Suppurative cervical osteomyelitis/ meningomyelitis
4	+ aggressive, star-gazing, unable to fly	-	mild	ABLV encephalitis
5	-	-	moderate	Trauma
6	+ paresis, paralysis, glassy eyes	-	mild	ABLV encephalitis

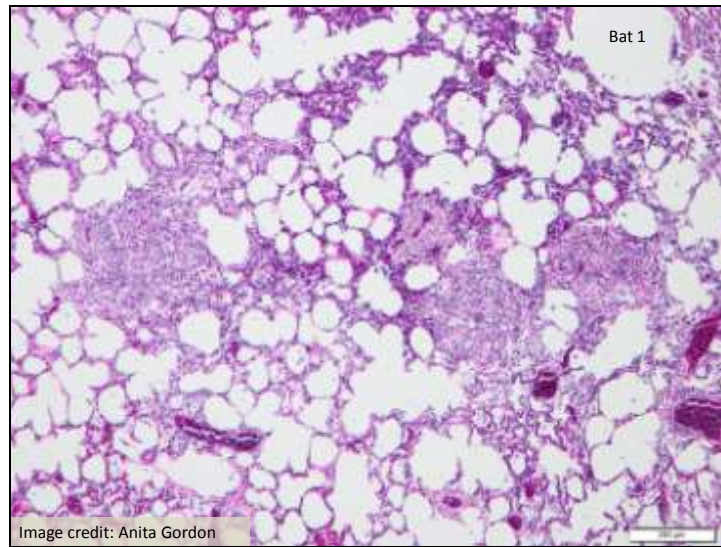
Slide credit: Anita Gordon

Slide 7



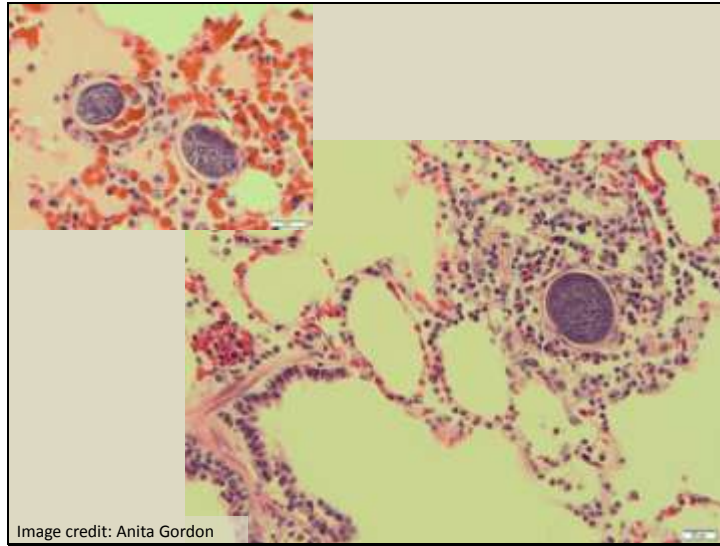
Increased cellularity (oedema and congestion may be related to euthanasia)

Slide 8



Granulomatous pneumonia

Slide 9



Schizonts in blood vessels

Slide 10

• Variable schizont shape: round, oval, fusiform, sinuous, irregular

• Some similarities to *Johnsprentia copemani*, described from lungs of black FF¹

¹ Landau I, Chavatte JM and Beveridge I (2012). *Memoirs of the Queensland Museum* 56(1): 61-66.

Bat 3

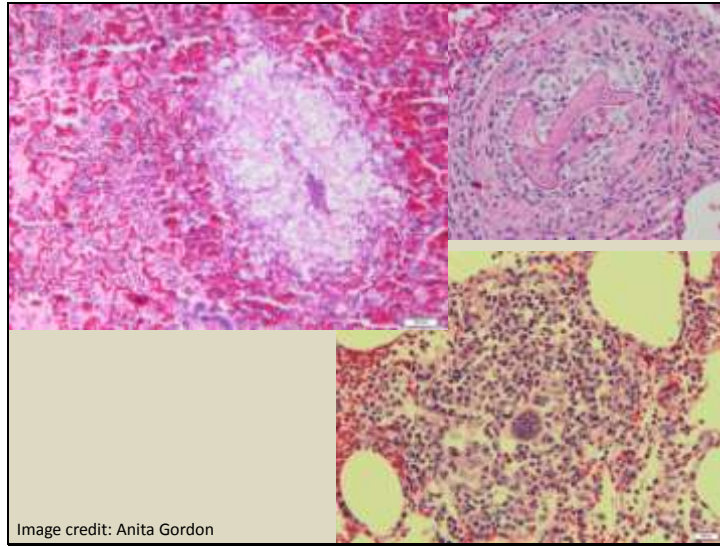
Bat 5

Bat 6

Bat 2

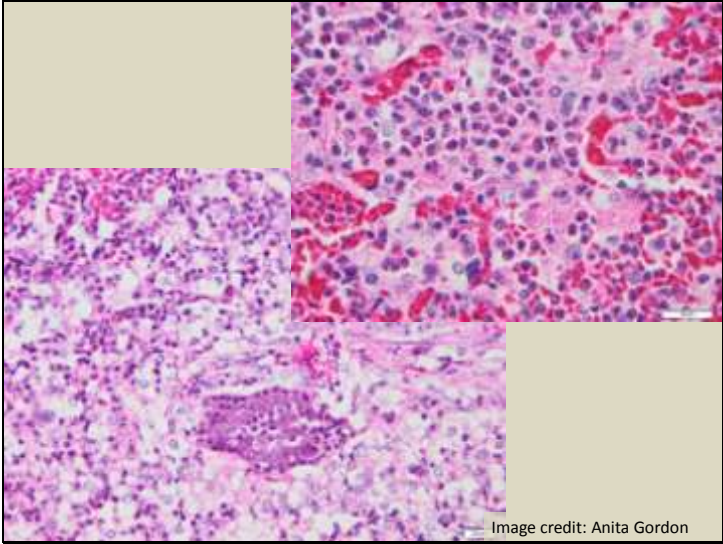
Image credit: Anita Gordon

Slide 11

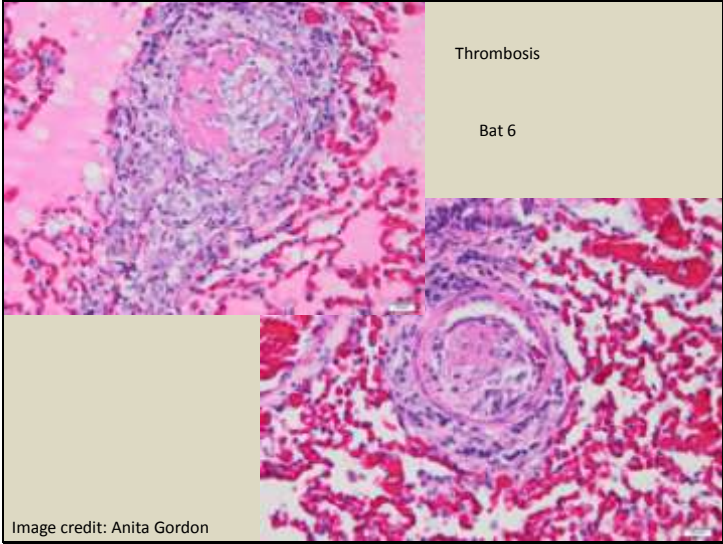


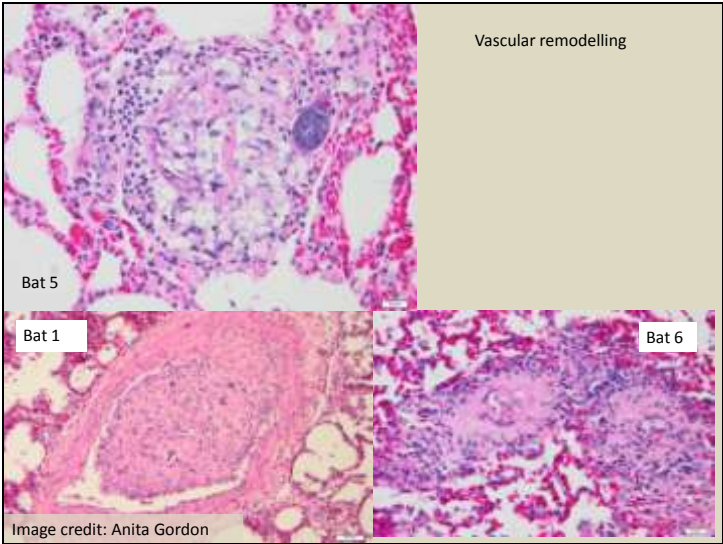
Degenerating schizonts

Slide 12

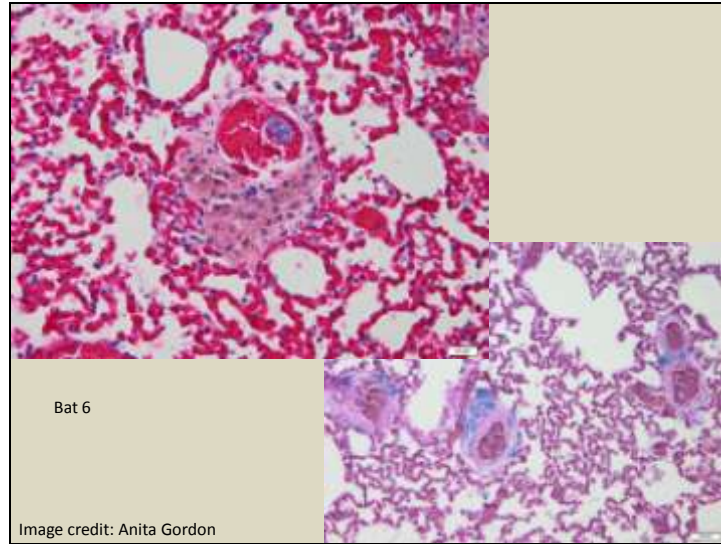


Eosinophilic component of pneumonia





Slide 15



Haemosiderin laden macrophages (iron stains with Prussian Perl's Blue)

Cytochrome b PCR of fresh tissues from LRFF

Case	Tissue tested	Histological evidence of haemosporidial schizonts	PCR result
A (bat 6)	lung	+	+
B	lung	+	+
	liver	Multifocal granulomas	+
C	lung	-	+

A 486bp portion of the cytochrome b (cytb) gene was amplified by PCR and sequenced¹. 99% identity was obtained with a parasite described as "*Hepatocystis* sp." from *Pteropus vampyrus* in Malaysia.

PCR may be detecting erythrocytic stage, not schizont

¹. Olival KJ, Stiner EO and Perkins SL (2007). *Journal of Parasitology* 93(6):1538-1540.

Slide credit: Anita Gordon

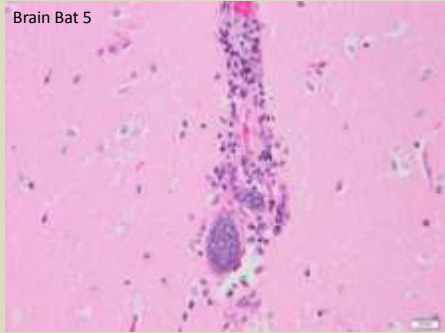
Further Molecular Testing

- March 2016: Commenced collaborative project with Macquarie University and Max Planck Institute for Infection Biology, Germany
- Tissues (lung, liver) and/or blood from much larger numbers of Qld FF
 - most clinically normal
 - subset that have had full necropsy: LRFF ($n = 7$), BFF ($n = 1$)
- For LRFF PCR of four genes: cytochrome b (cytb), apicoplast (clpc), cytochrome oxidase (cox1) and nuclear elongation factor 2 (ef2)
- PCR detects infections
 - in histologically negative animals
 - in blood-smear negative animals
- **Preliminary sequencing analysis shows that all parasites identified belong to genus *Hepaticystis***
 - parasites found in all 3 *Pteropus* spp. (LRFF/BFF/SFF)
 - individual bats host different parasite haplotypes or even species
 - likely that PCRs are detecting erythrocytic and/or tissue stages

Slide credit: Anita Gordon

Tissue Specificity?

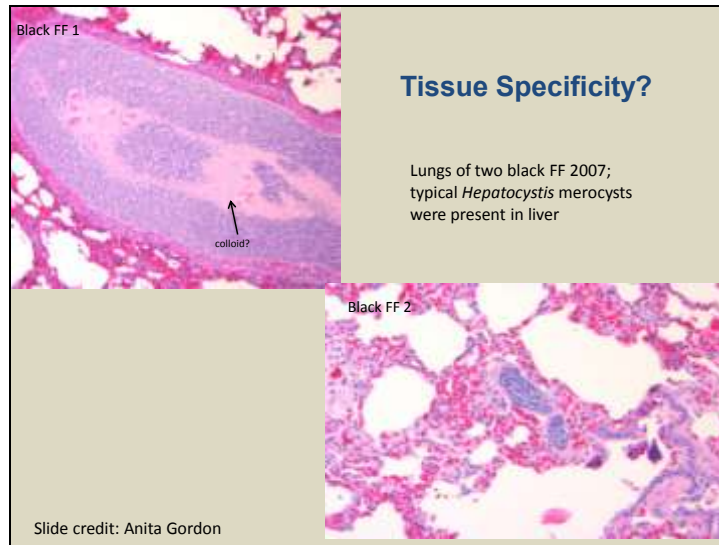
Brain Bat 5



Tissue location of schizonts has been used as a taxonomic feature for pteropid haemosporidia

Landau *et al.* (2012). *Parasite* **19**:137-146.

Slide credit: Anita Gordon



Black FF 1

Tissue Specificity?

Lungs of two black FF 2007;
typical *Hepatocystis* merocysts
were present in liver

colloid?

Black FF 2

Slide credit: Anita Gordon

Conclusion:

- Taxonomic clarification underway
 - Combined morphological/molecular approach
- Subclinical haemosporidial infections appear to be widespread in Qld FF
- Tissue schizonts can be associated with significant pathology in individual LRFF
 - Granulomatous, eosinophilic pneumonia with vasculitis

Slide credit: Anita Gordon

Acknowledgements

Update on haemosporidial pneumonia in little red flying foxes (*Pteropus scapulatus*)

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