Evaluation of methods for extraction of DNA from *Encephalitozoon cuniculi*

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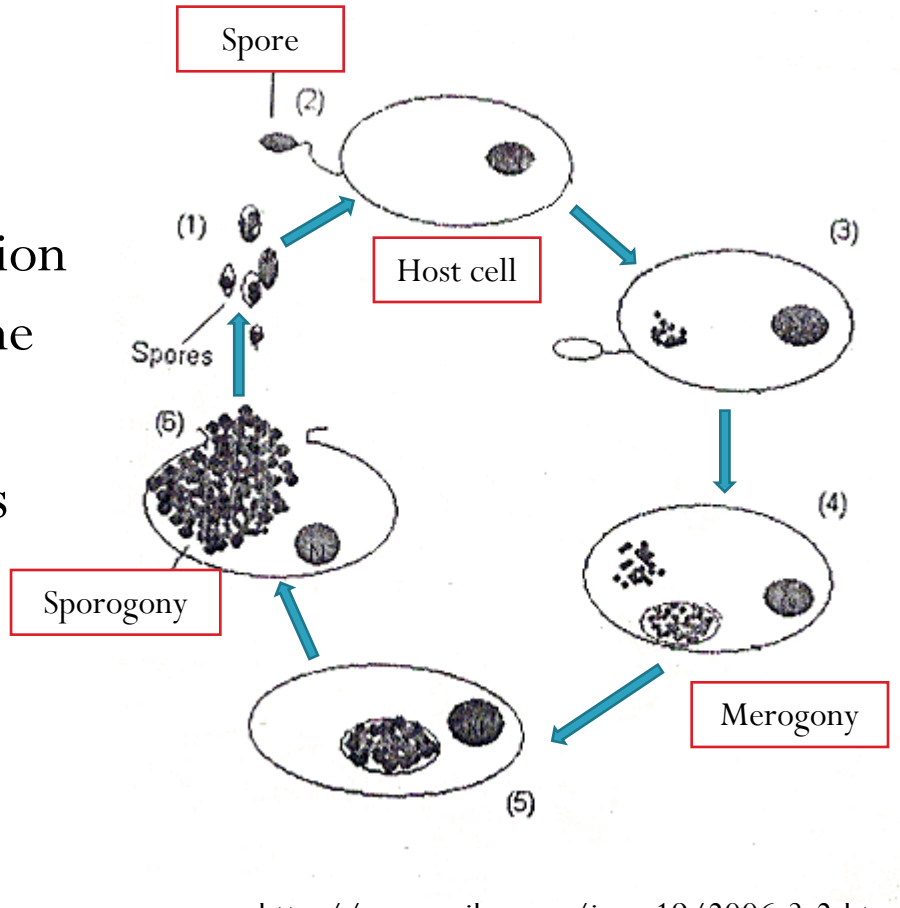
Encephalitozoonosis

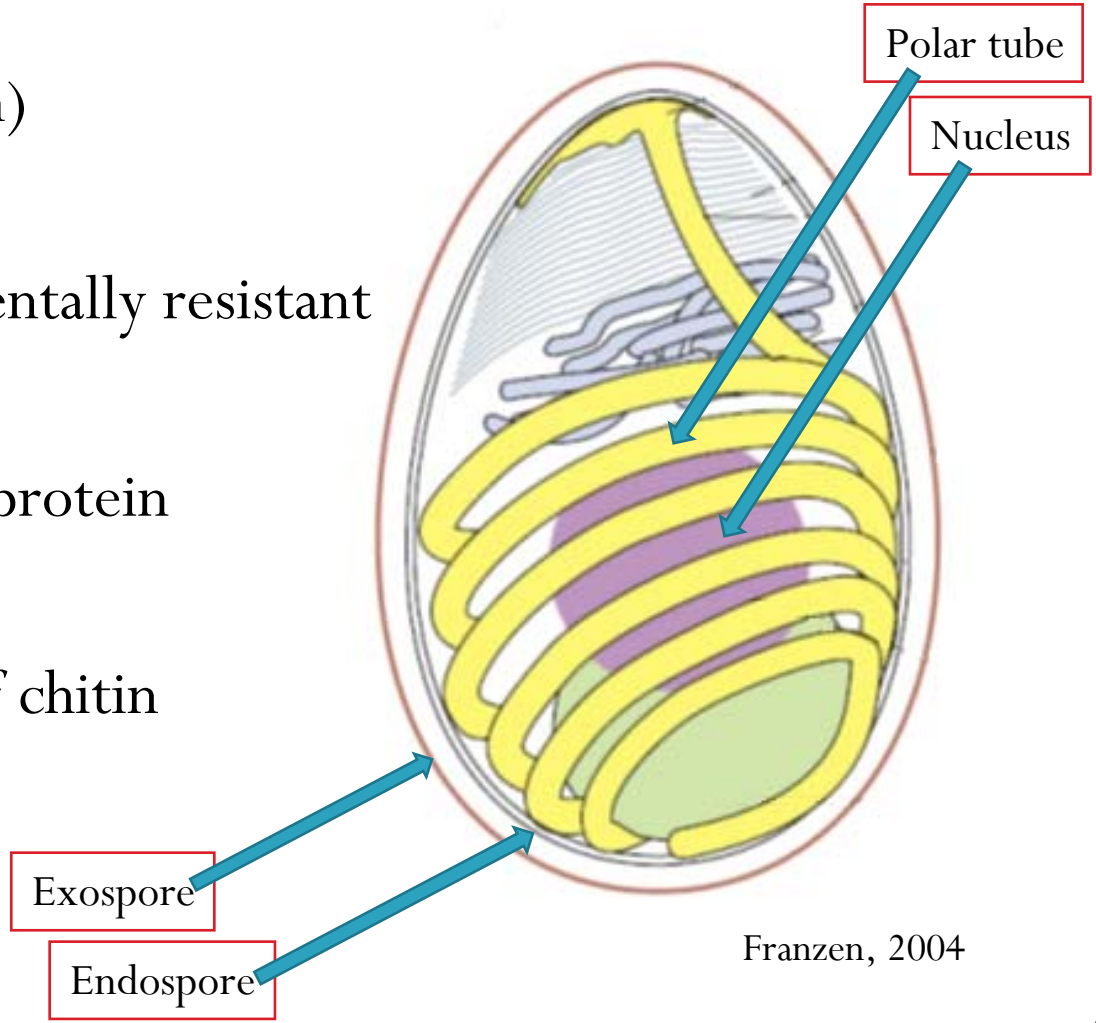
- Disease caused by *Encephalitozoon cuniculi*
  - Fungi (Microsporidia)
  - Unique mechanism of invasion
  - Infective spores shed in urine
- Causes subclinical infections and severe disease
- Zoonotic threat to immunocompromised



http://www.ojhas.org/issue19/2006-3-2.htm

Structure of *E. cuniculi* Spores

- Very small (1.5-3 µm)
- Spores are environmentally resistant
- Exospore consists of protein
- Endospore consists of chitin



Franzen, 2004

Diagnostic Techniques

Diagnostic Technique	Limitations
Clinical Presentation	- Non-specific signs - Differential diagnosis
Serology	- Indicative of exposure only
Cell Culture	- Time consuming
Microscopy	- Spores may be excreted in small numbers - Small size of spores makes identification difficult

PCR

- Potential to be a rapid and highly sensitive method of detection
- Under-developed in veterinary medicine
- Concerns regarding the sensitivity
  - Moderate sensitivity reported in literature
  - Intermittent shedding of spores or vs. inadequate assay design

DNA Extraction

- No standardized method of DNA extraction
- Sensitivity of PCR depends on the quality and quantity of DNA extraction
- Must first disrupt thick spore to extract DNA
- Enzymatical disruption
- Mechanical disruption

Objectives and Hypothesis

- Objective: To assess the efficacy of different DNA extraction methods for preparation of DNA for PCR

- Hypotheses:
  1) A combination of enzymatical and mechanical spore disruption following by DNA extraction with commercially available DNA extraction kits will provide sufficient quantity of DNA to be used for PCR

  2) There will be variation in the quantity of DNA extracted amongst the DNA extraction methods

Materials and Methods

- Preparation of spores:
  - Using a reference strain of *E. cuniculi* grown in cell culture
  - Purified and counted (100,000 spores per sample)
  - Enzymatical, mechanical or combination disruption of spores

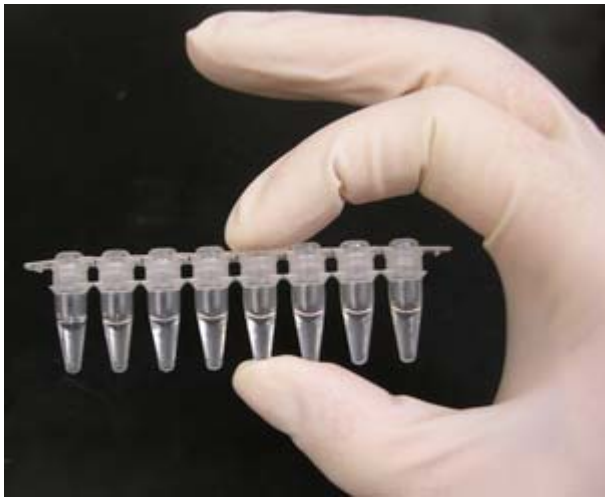
- DNA extraction:
  - Using one of six commercial kits
  - Samples performed in triplicate
  - Quality and quantity measured using spectrophotometry

Extraction Kits Tested

- PrepGEM™ (Zygem)
- DNAzol® (Invitrogen)
- Maxwell® 16 Tissue DNA Purification kit (Promega)
- High Pure PCR Template Preparation Kit (Roche)
- DNeasy Plant Mini Kit (Qiagen)
- QIAamp® DNA Stool Mini Kit (Qiagen)

Materials and Methods

- PCR analysis:
  - Using *E. cuniculi* specific primers
  - 50 ng of DNA per reaction
  - Quality of DNA assessed using agarose gel electrophoresis



Results – Experiment #1

- Mean DNA concentration (ng/µl) ± SD:

	DNeasy	Maxwell	QIAamp	High Pure	DNAzol	PrepGEM
No treatment	2.18 (±0.35)	2.85 (±0.03)	7.56 (±1.58)	9.87 (±4.35)	1.67 (±2.69)	8.27 (±10.31)
Mechanical	1.94 (±0.50)	3.67 (±1.57)	4.66 (±1.13)	12.81 (±5.83)	1.47 (±5.92)	5.00 (±4.11)
Enzymatical	3.18 (±0.68)	3.79 (±0.94)	5.59 (±1.21)	11.55 (±0.98)	1.66 (±1.40)	**88.44** (±4.11)
Combination	2.91 (±2.12)	4.10 (±0.94)	4.42 (±0.71)	12.68 (±1.38)	1.41 (±2.50)	**91.74** (±3.63)

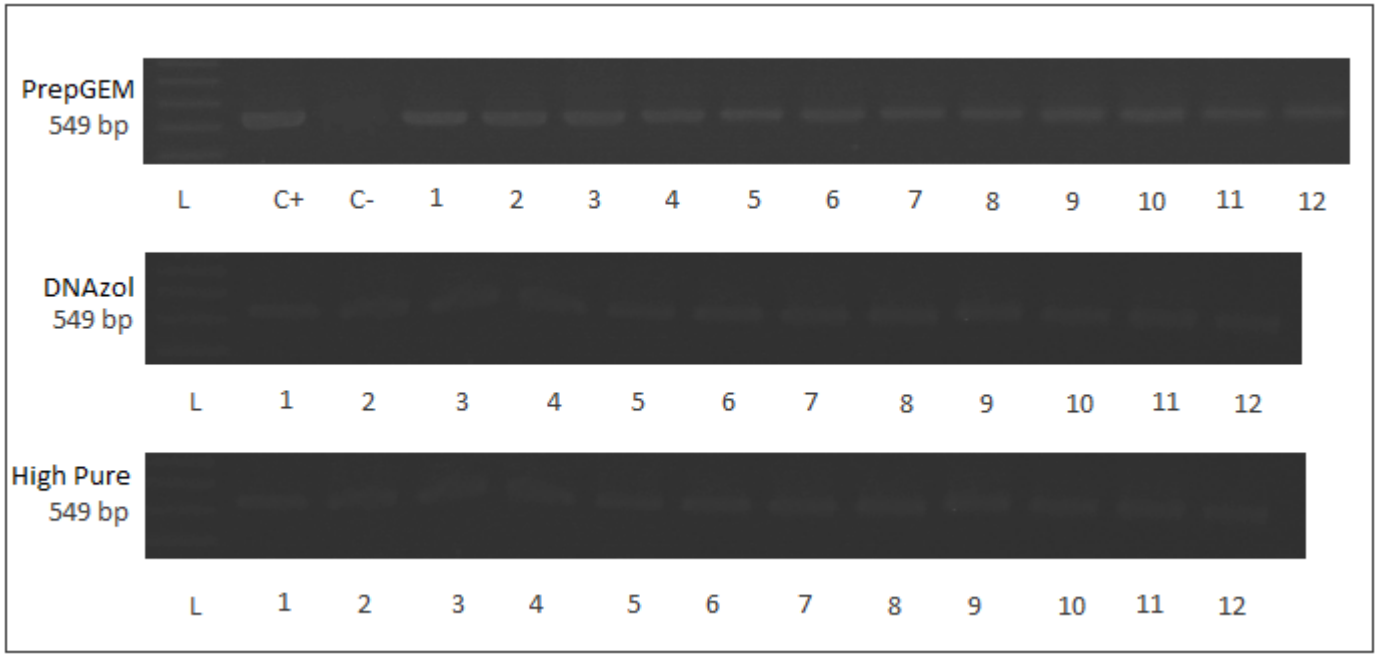
Results – Experiment #2

- Mean DNA concentration (ng/µl) ± SD:

	High Pure	DNAzol	PrepGEM
No treatment	10.25 (±2.93)	3.47 (±1.21)	4.16 (±1.49)
Mechanical	14.70 (±3.71)	0.79 (±0.13)	12.41 (±1.93)
Enzymatical	8.35 (±1.96)	1.69 (±0.82)	**148.36** (±2.56)
Combination	23.60 (±20.08)	1.25 (±0.78)	**161.63** (±0.52)

Results – Experiment #2

- Agarose gel electrophoresis:



L= ladder, C+= positive control, C–= negative control, 1-3= no treatment, 4-6= mechanical, 7-9= enzymatical, 10-12= combination

Overall Comparison

	Extraction Efficiency	Quality (OD ratio)	Processing Time (m:s)	Difficulty of Procedure	Cost Per Sample ($)
DNeasy	0.05	2.80	42.10	Difficult	3.56
Maxwell	0.07	1.10	45:40	Easy	5.38
QIAamp	0.11	2.11	34:20	Difficult	3.54
High Pure	0.24 (0.17)	1.39 (1.54)	17:10	difficult	3.19
DNAzol	0.21 (0.02)	3.47 (1.20)	13:10	Moderate	2.45
PrepGEM	1 (1)	0.81 (0.81)	12:30	Easy	1.40

Extraction Efficiency = average yield of kit / average yield of best kit

A_{260}/A_{280} of pure DNA = 1.8-2.2

Conclusions

- A combination of enzymatical and mechanical disruption followed by DNA extraction using PrepGEM produced the highest quantity of DNA
- There is considerable variation in the quantity of DNA extracted amongst the DNA extraction methods tested
- Current methods for extraction of DNA from *E. cuniculi* need to be further evaluated

Future Studies

- Development of a Real time PCR assay
- Detection threshold study

Thank-you