The challenge of making effective multicellular parasite vaccines

Professor Jacqui Matthews BVMS PhD MRCVS

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Multicellular parasites

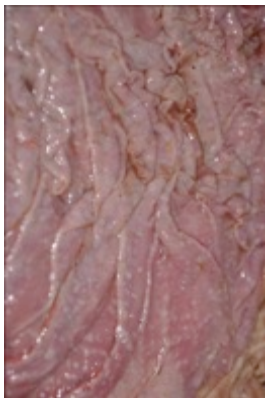
- Major threat to health & welfare

- Disease
- Reduce food quality
- Reduce profitability

- Antiparasitics global market $3B

Helminths

- Nematodes
- Trematodes
- Cestodes



Costs difficult to calculate

Example: fluke in cattle

- Reduced milk yields, lower reproductive performance
- Beef cattle: extra 30-80 days to finish

- EBLEX: £25-30/head = £8-9.5M/year to beef industry
- Swiss study: beef & dairy - 299€ per animal
- Liver condemnation: as high~60%

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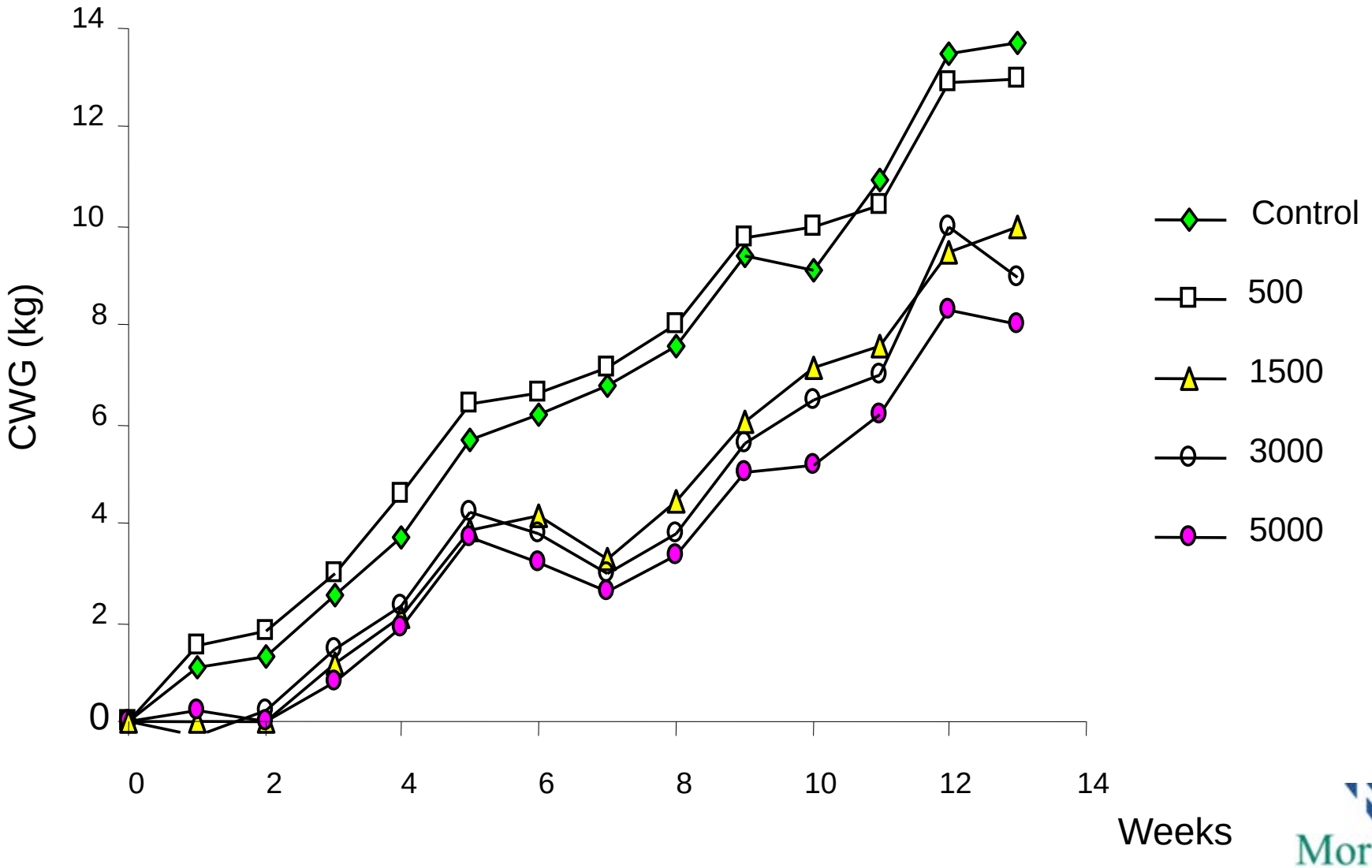
Nematode infection in lambs

Teladorsagia circumcincta



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Anthelmintics

Primary method of control

Broad spectrum products available for 50 years

These products have set the benchmark (95-99% efficacy; multivalent)

Inexpensive

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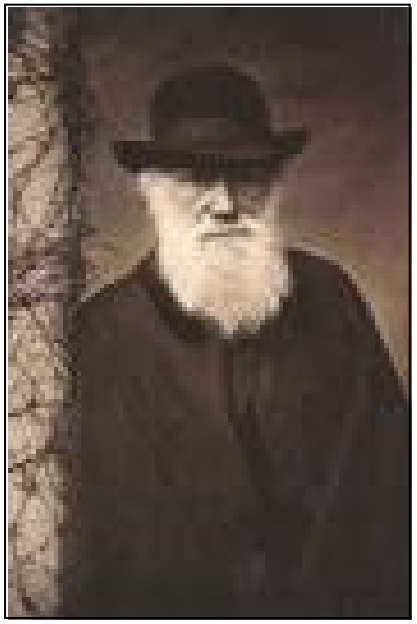
Anthelmintic resistance

- Helminth populations
  - extremely large
  - genetically very diverse
  - adapt under selection pressure

- Drug treatment potent trigger for adaptation in response to selection pressure



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Resistance index: nematodes

Relatively rapid resistance to all classes

Widespread resistance to 1st three classes

Widespread resistance to some classes

Increasing reports to all classes

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Problem: highly prevalent pathogens, drug resistance rife, less investment by pharma in drug discovery

Solution: develop vaccines

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How good do nematode vaccines need to be?

- Unlike microbial vaccines, sterile immunity not always essential
  - Where does this leave Proof of Concept?
- Tool to reduce pasture contamination
  - e.g. modeling: 80% reduction egg output in 80% sheep better than standard chemical control
- More works needs to be done in this area
  - 'optimal control'

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Approaches

- Attenuated vaccines – irradiated
- Fractionated ‘native’ antigens
- Vaccines informed by knowledge of host/parasite interaction
  - ES antigens
  - Surface antigens
  - Correlates of protection
- Variable success with ‘native’ antigens

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TPP?

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Recombinant vaccines

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Challenges

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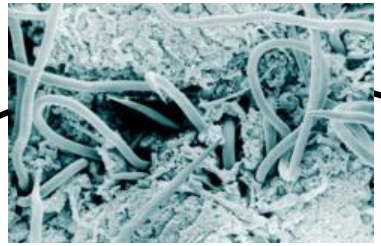
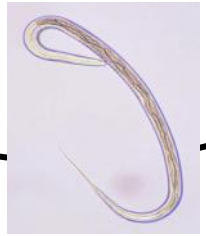


eggs excreted in dung

hatch and larvae move to pasture



ingestion from pasture

development in host

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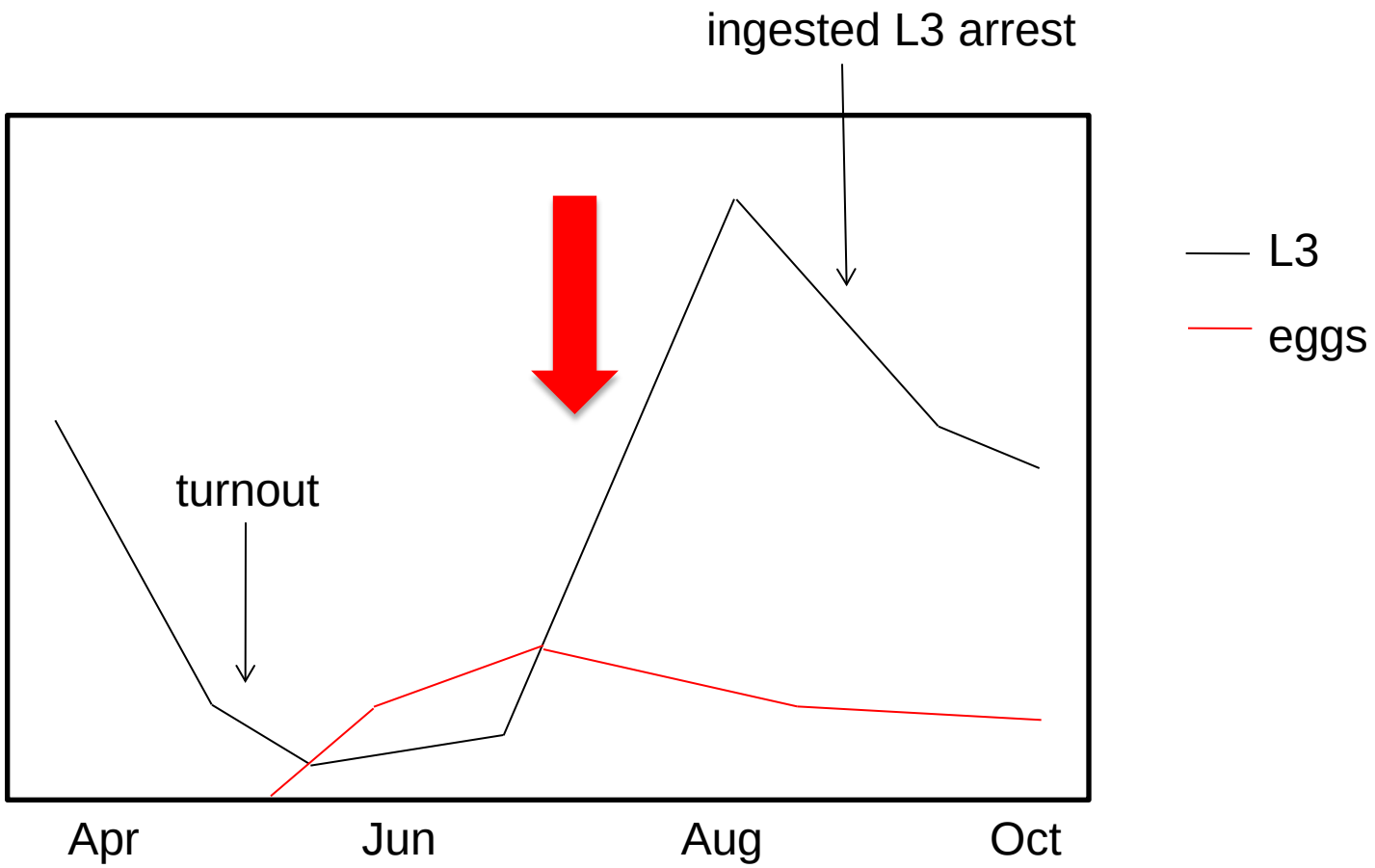
Immunity develops slowly



Dairy and autumn born beef calves

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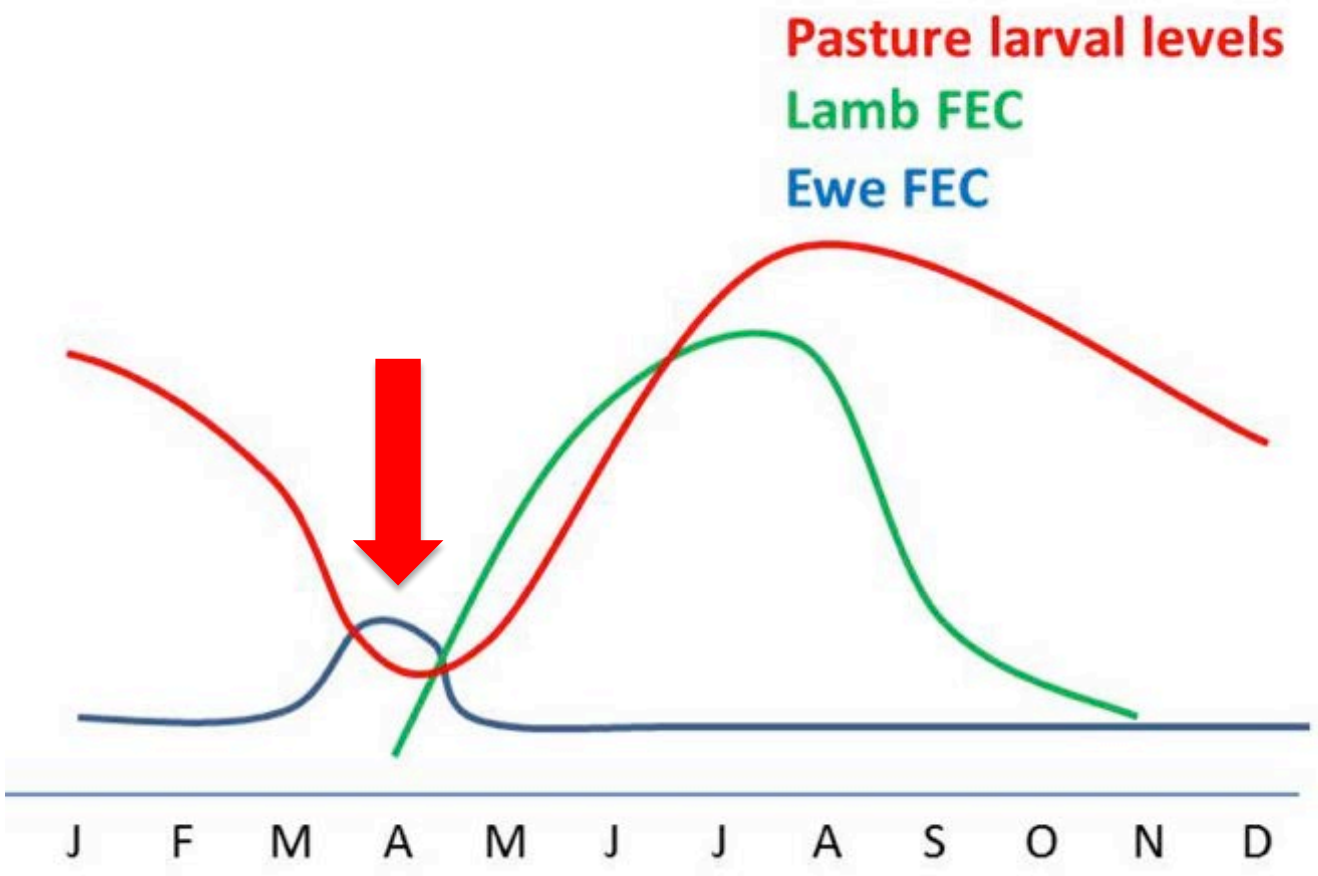
Immunity affected by pregnancy



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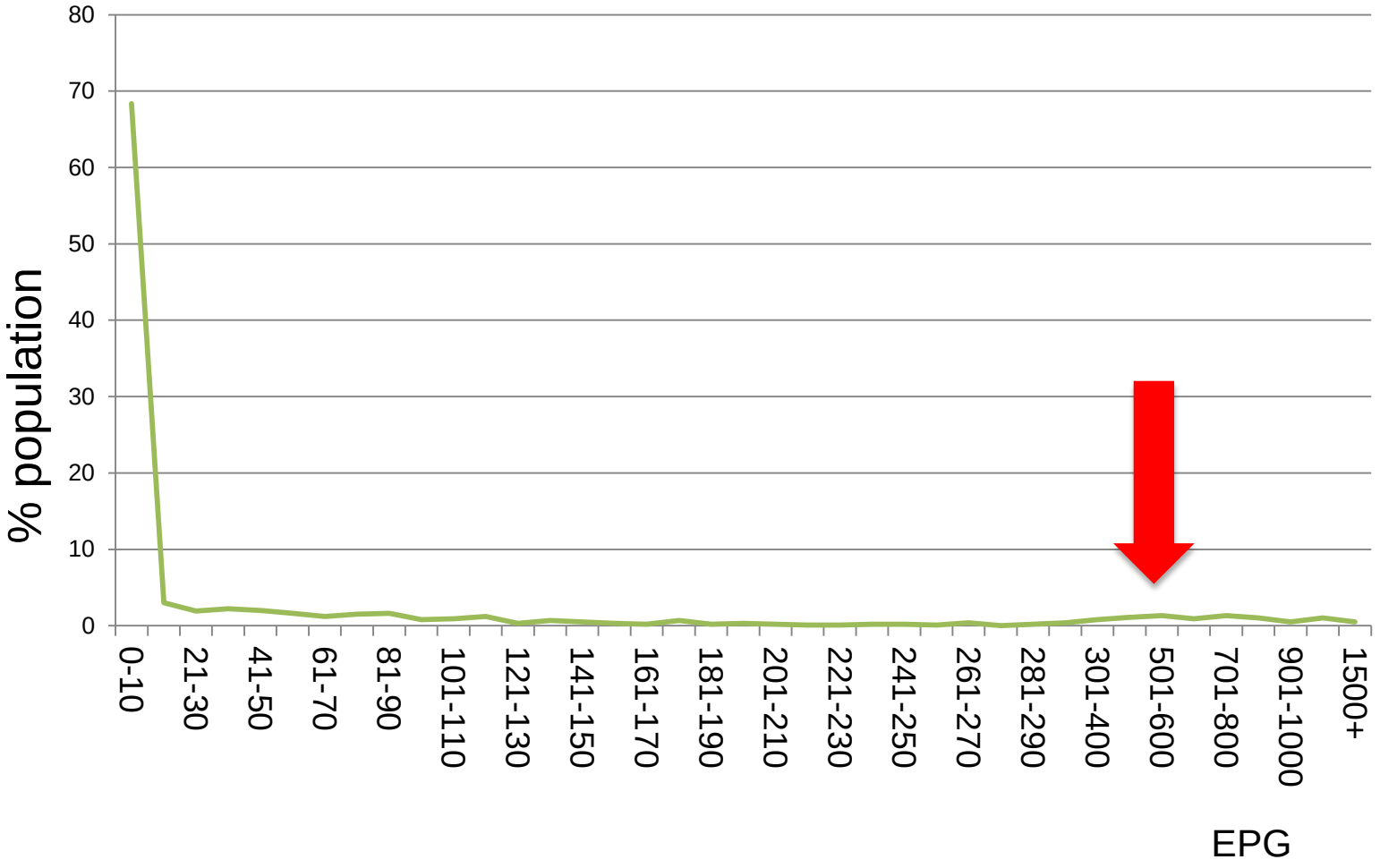
Negative bionomial

Some animals never respond well



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Complexity of the pathogen

Success with native antigen (mixes) does not equal success with recombinant versions of these

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Haemonchus contortus

- Sheep, goats, cattle, wild ruminants
- More prevalent in warm moist regions
- Acute disease can be fatal
- Anthelmintic resistance rife

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Native gut antigen approach

- Blood feeder
  - Purify gut antigen (5 ug) and immunise SQ with saponin (1 mg)
  - Consumed antibodies damage worm gut cells
- High levels of protection obtained in several trials
  - 80%+ reduction in FEC
  - 50%+ reduction in worm numbers

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Recombinant vaccine?

Proof of concept

Establishing protection

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H11

- Native complex of microsomal aminopeptidases
- Three active H11 isoforms expressed in baculovirus
  - No protection
- Active site domain expressed in *E. coli* as inclusion bodies
  - No protection
  - Other combos of *E. coli*-expressed isoforms not protective

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H-galGP

- Aspartyl & metallo proteases
  - H11-free preparations
  - Significant protection (not as high as H11)
  - Not able to express as soluble proteins in yeast and baculovirus
  - One recombinant MEP gave ‘limited protection’

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Recombinant cocktail

- Recombinant H11, H-gal-GP and thiol sepharose binding protein complex components combined
  - No protection obtained

- Protective native epitopes conformational (e.g. carbohydrate) in nature?
- Redundancy of function between enzyme classes in worm gut?
- Cloned enzymes not protective components?

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Barbervax

- Lambs: 5 SQ 1 ml injections ~6-wk intervals* (TPP!)
  - currently registered for use in lambs only
- 1st 2 vaccinations do not protect, only after 3rd
    - lasts for at least 6 weeks
- Further vaccinations needed/6 wks in risk period
  - non-responders
- After course, a single boost in subsequent season provides 6 wks' protection

* http://barbervax.com.au/how-to

Final product: 5-pack course $3 a head

Developed and marketed by Moredun

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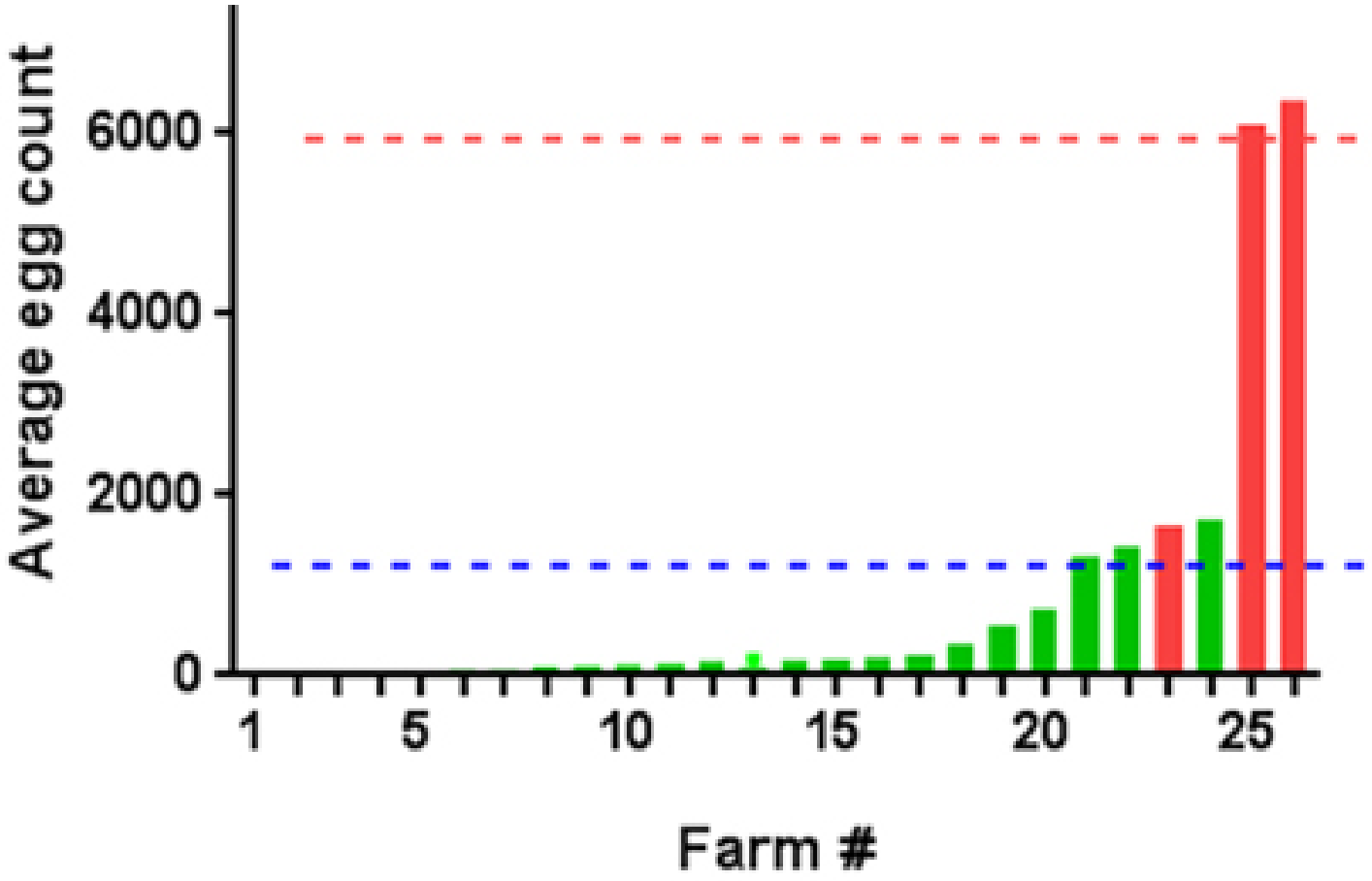
~50,000 lambs on 35 NSW properties 2014-15



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Wormboss ('end users') say……

Pros

1. No meat withdrawal period
2. Good tool where parasite 'out of control' and farmer has to drench regularly
3. Option where multidrug resistant isolates present
4. Fits Wormboss programme with grazing, breeding tools

Cons

1. Not one-shot
2. Protection short lived (resource)
3. Annual boost
4. Not complete alternative to drenching; treatments required (for other spp.), FEC monitoring (may decrease over long term use)

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Ostertagia & *Cooperia* native vaccines

- High global prevalence
- Substantial production losses in beef and dairy cattle
- Several experimental native vaccines tested vs. *O. ostertagi*
- Most promising = ASPs from thiol purified adult ES
    - 55-62% reductions after experimental trickle challenge
  - ASPs identified as promising antigens in *C. oncophora*

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Native ASP from *Cooperia*

- DD ES ASP
  - Pen trial. 3 x V IM Quil A. Trickle challenge. Vaccinates significant reduction (91%) cum FEC. Significantly higher inhibited L4. Adult worms smaller
  - Field trial. Same regime: natural challenge. FEC in vaccinates reduced across grazing. Significant reduction in the cum FEC (58%). 65% reduction in mean pasture L3 at housing. Significant reduction (81.6%) in total worms

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Recombinant vaccines

FEC in calves vaccinated with recombinant *O. ostertagi* ASP1 expressed in insect cells or *P. pastoris* not significantly less than controls

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Challenges

Glycans

- Glycan engineering – helminth-like glycans.
- Expression in *C. elegans* (did not work with H11)

Protein folding

- MS and other methods to identify subtle differences in protein structure between native & recombinant proteins

Identify protective components of immune response in native vs. recombinant vaccinated calves

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Basing antigen selection on knowledge of the parasite and the host/parasite interface

Skip the native step?

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Teladorsagia circumcincta

- Primary pathogen temperate areas
- Estimated UK cost >£80M/year
- Anthelmintic resistance rife
  - monepantel resistance in 2 years
- Infected animals develop immunity
  - slowly
  - at a variable rate & level

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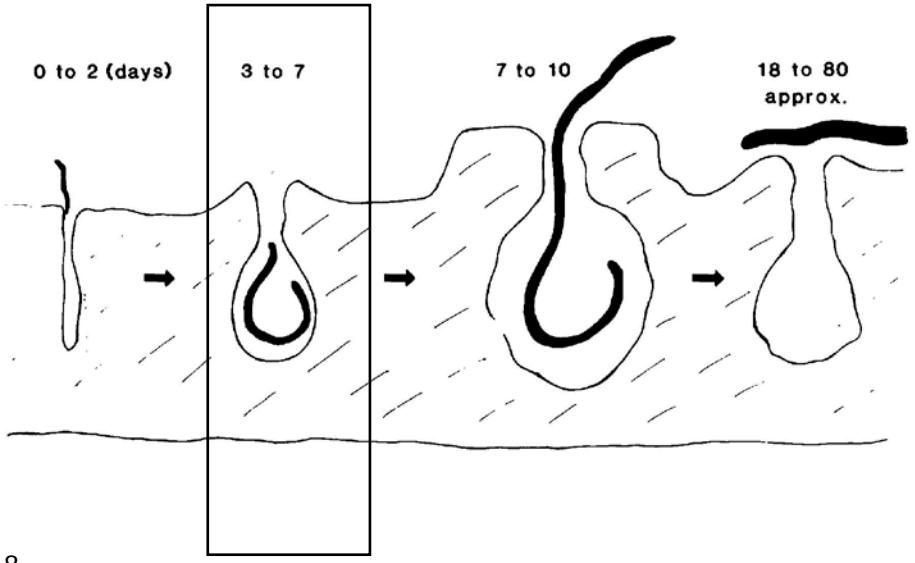
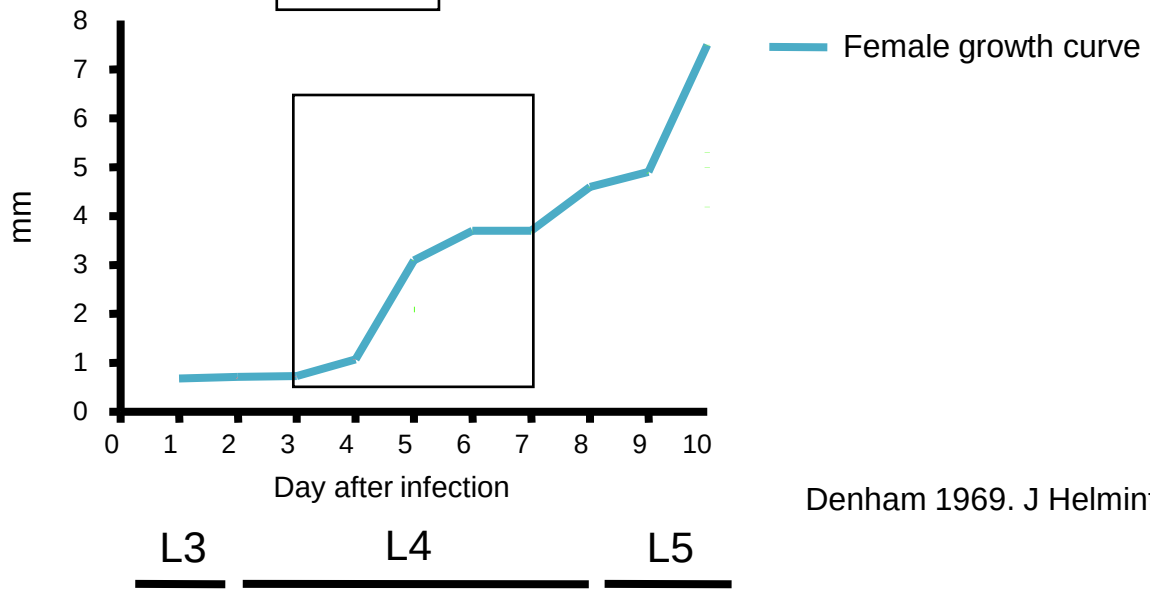
Strategy for mining antigens





Denham 1969. J Helminthol. 43:299-310.

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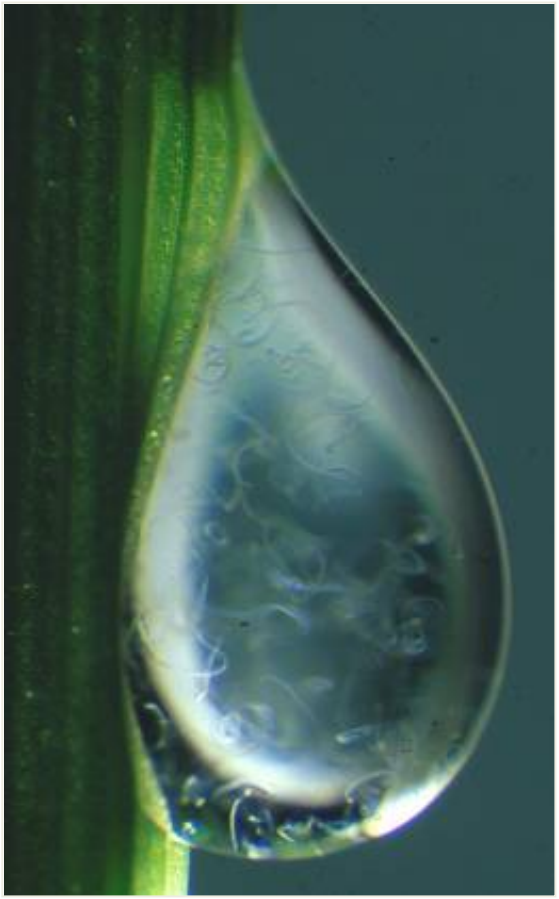
Immune correlates

- Local response
- Adoptive transfer
- Mucus & efferent lymph IgA
- Effects on developing L4



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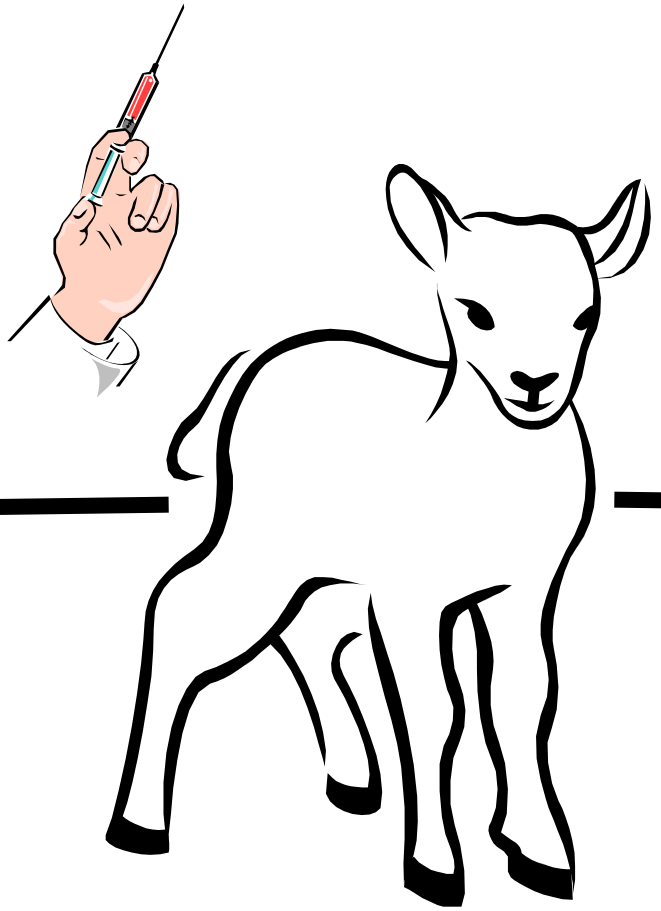
Prototype development in definitive host



What factors regulate immunity?

Which antigens stimulate immunity?

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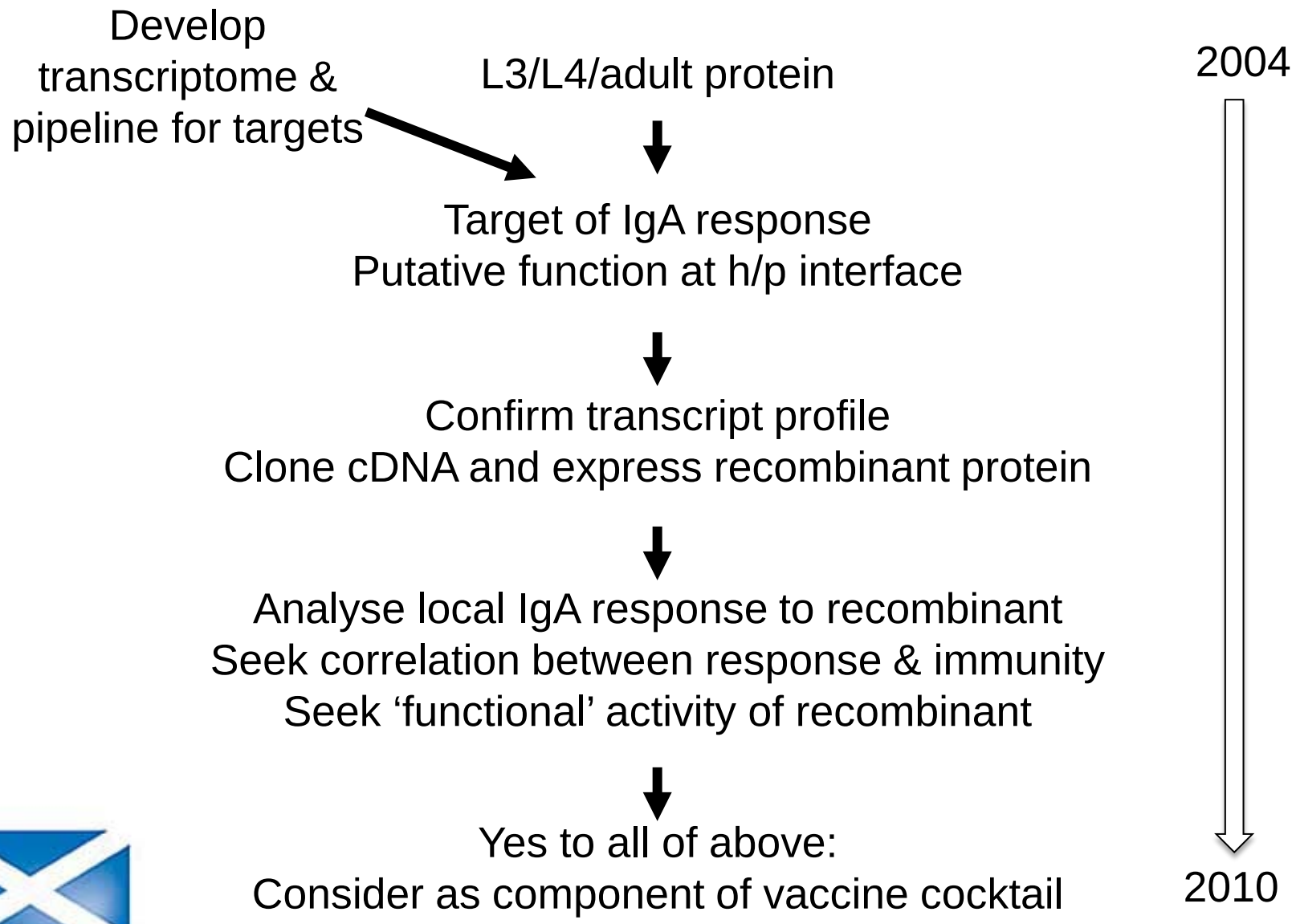

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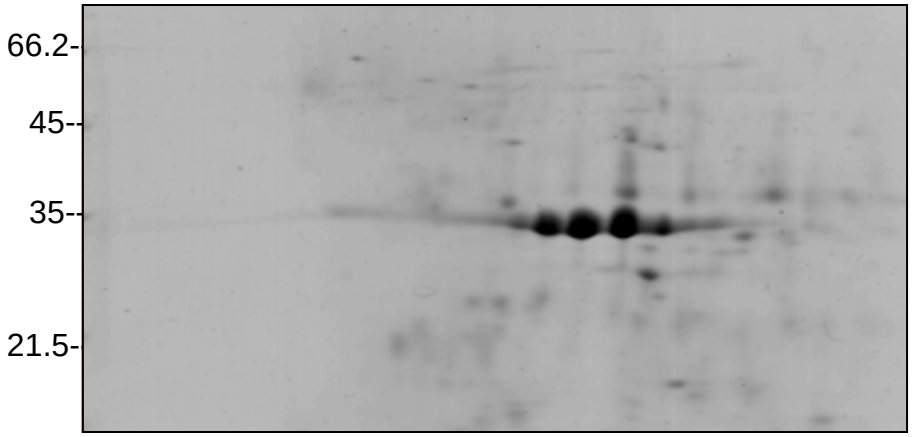
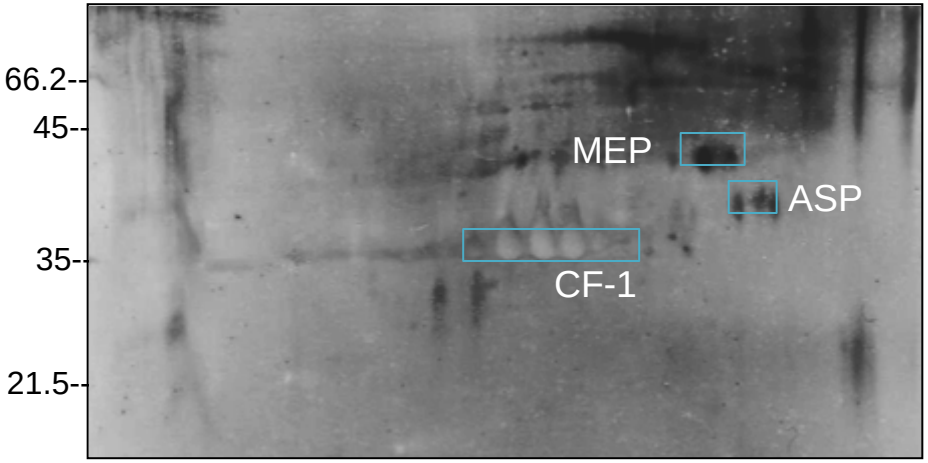
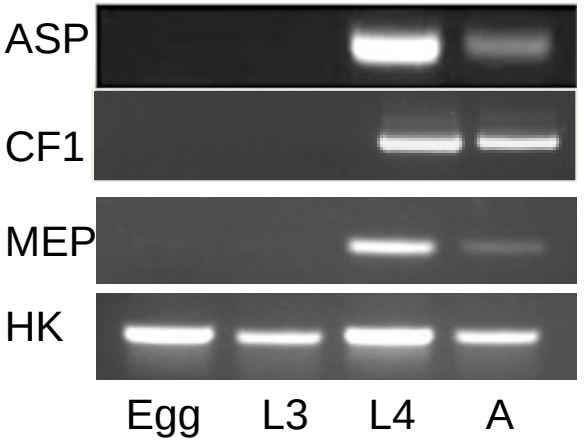
Colloidal Coomassie (5 dpi)



IgA: immune ewes





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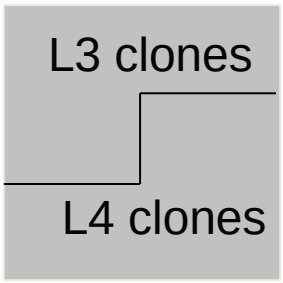
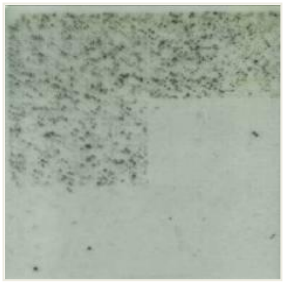
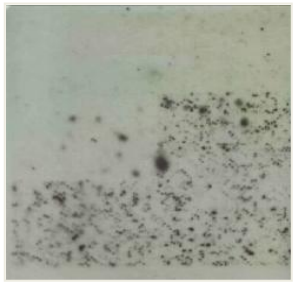
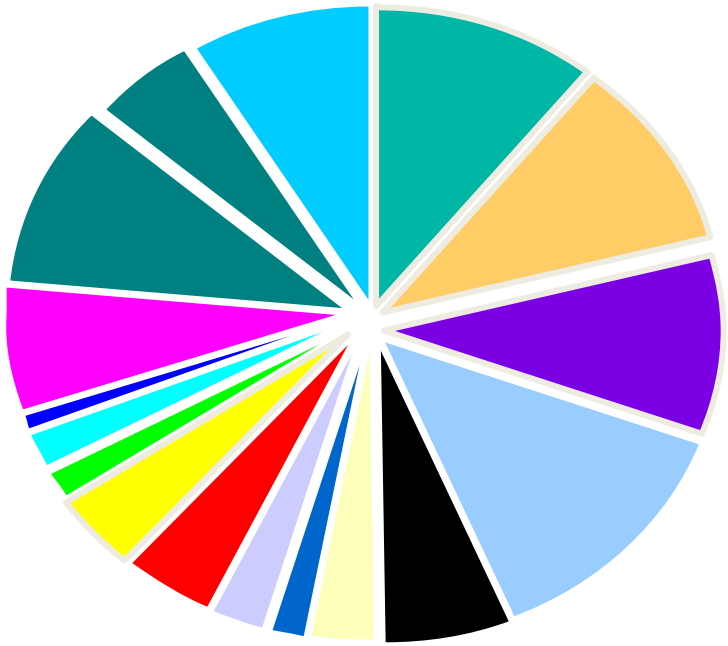
Suppressive subtractive hybridisation



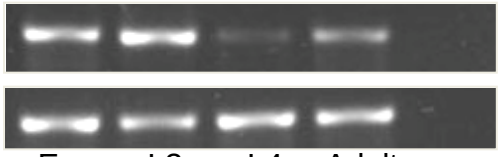




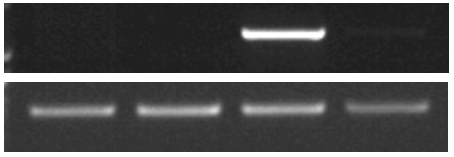


- **Tci-cathepsinF-1**
- other **proteinases**
- **ASPs**
- collagens
- globins
- protein metabolism
- lipid metabolism
- other metabolic
- muscle and cytoskeletal
- **'ES' proteins**
- lectins
- cell/cell interactions
- ribosomal
- other proteins known function
- unknown function/hypothetical
- no homology

MIF



APY



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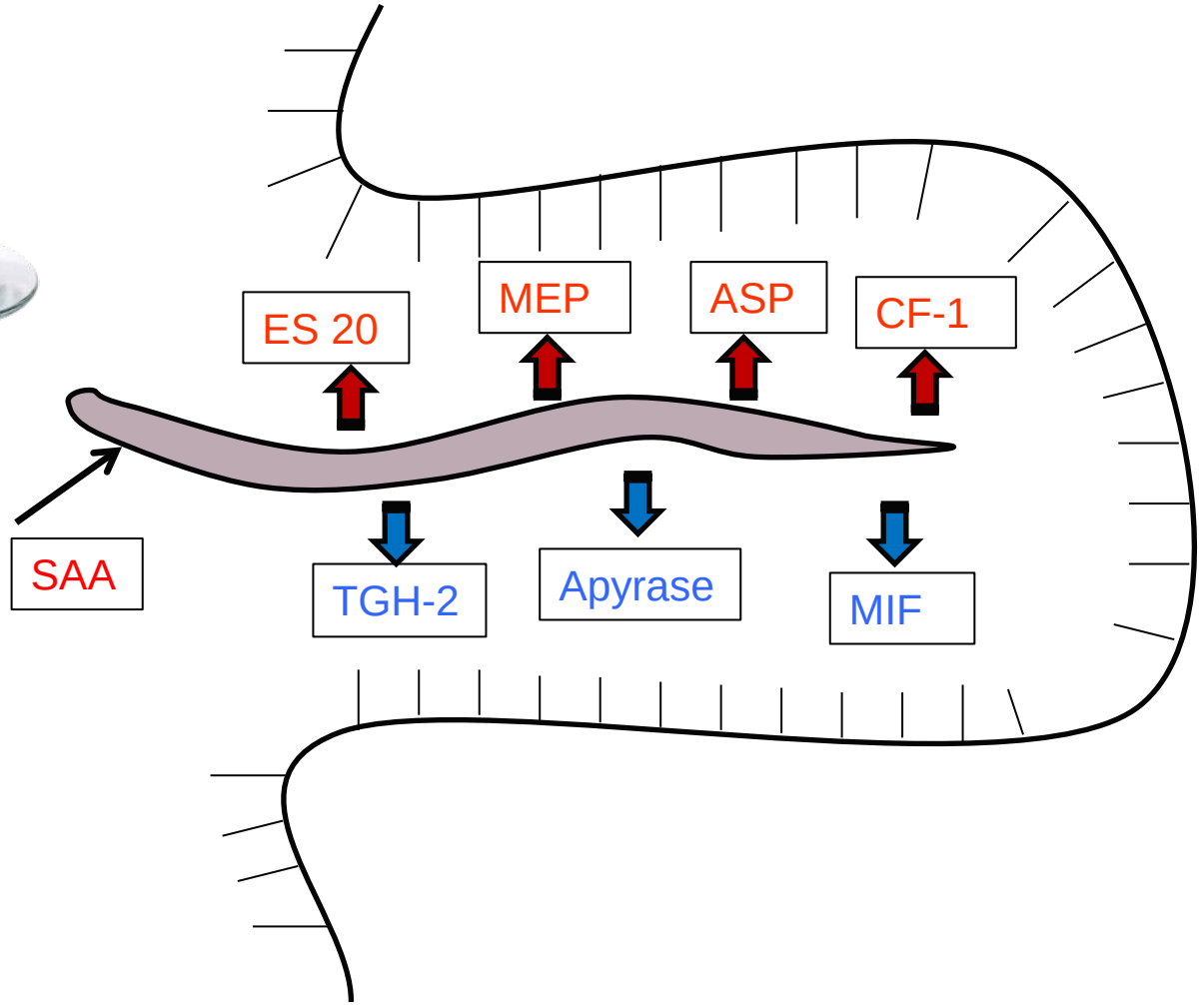

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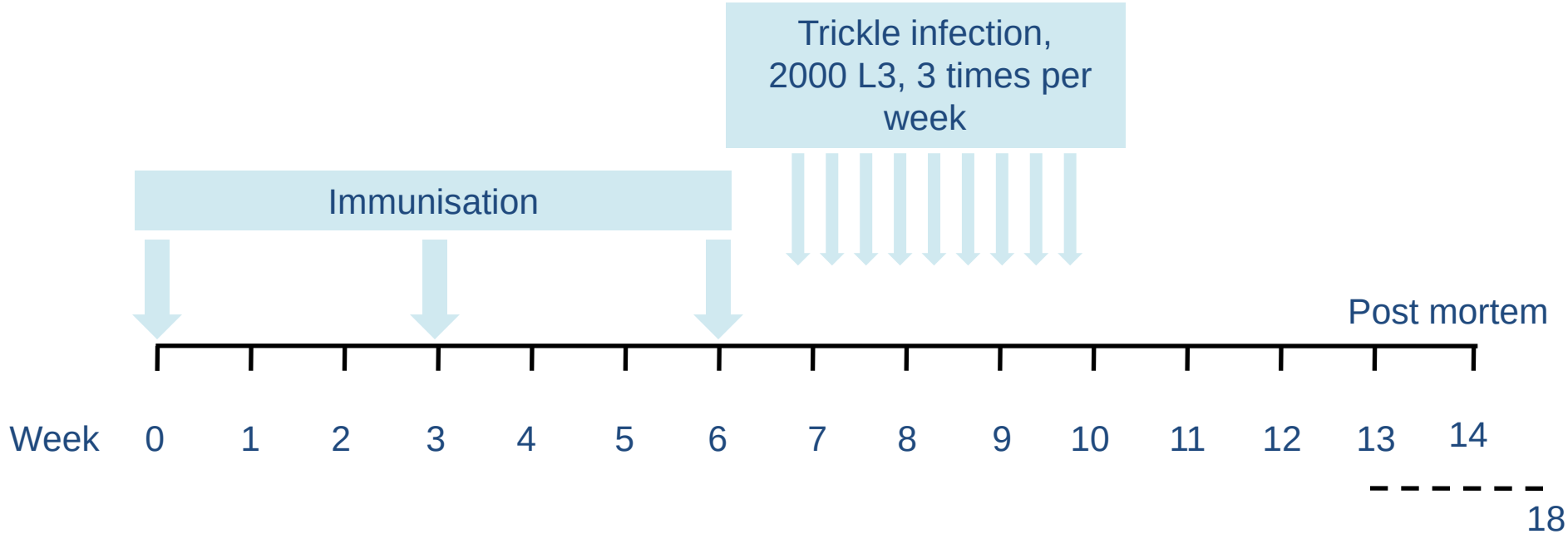
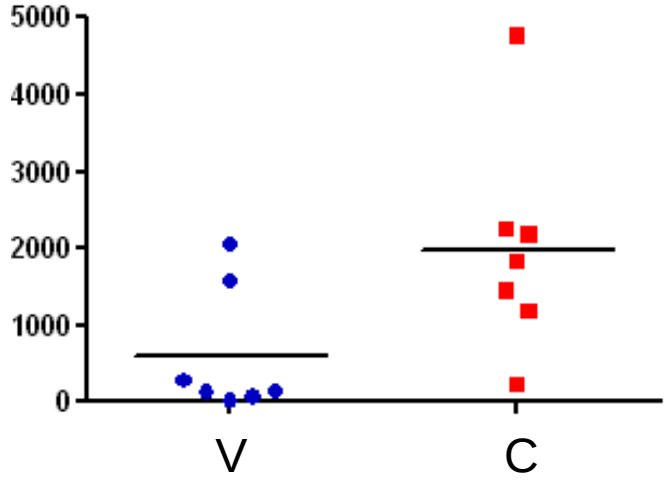
Trials: 2010-2015



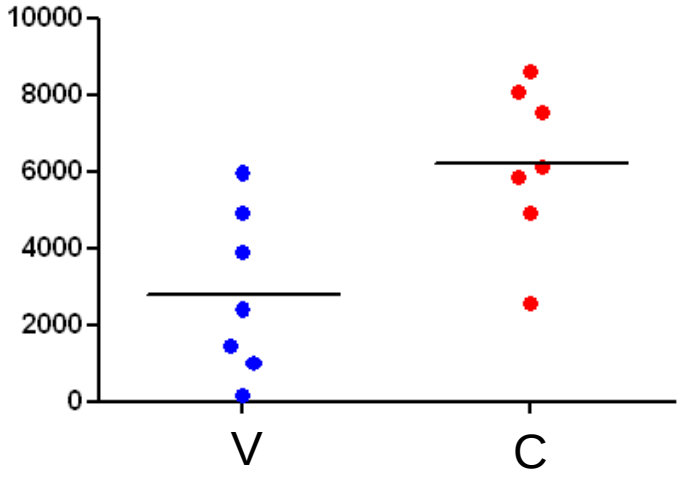
Serum/mucosal antibody, FEC, nematode burden, nematode length

Proof of concept

Cum FEC



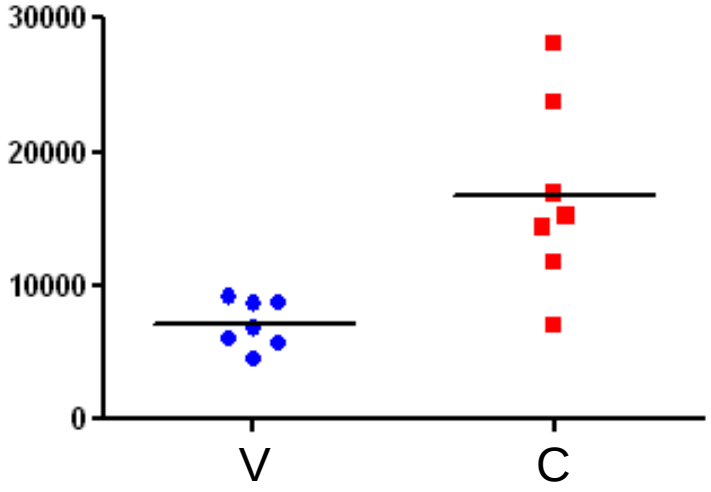
Adult burden



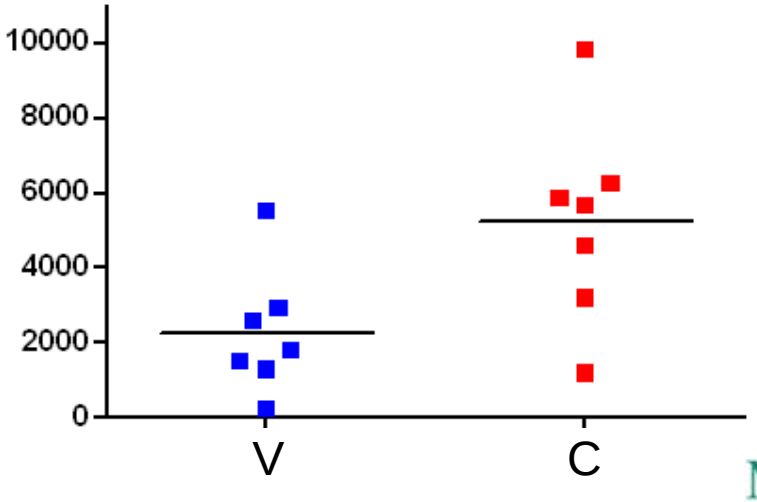
Trial 2

challenge to PM extended

Cum FEC



Adult burden



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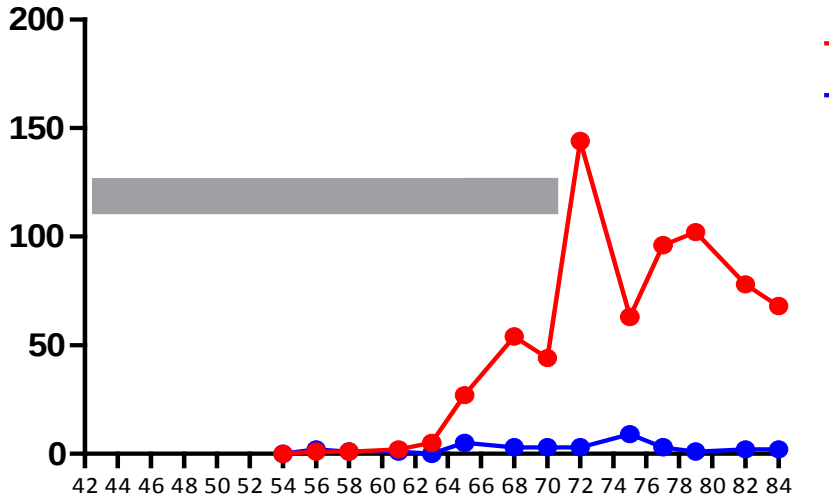
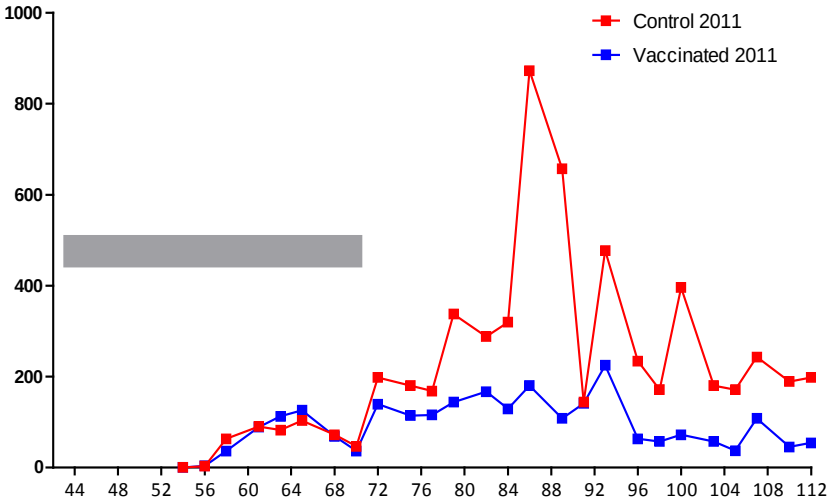
FEC pattern

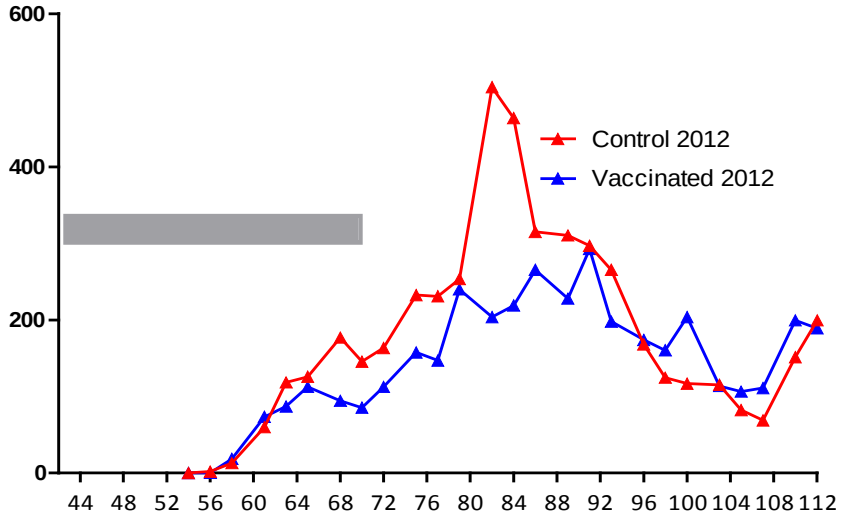
6.8 mo



5.8 mo



4.7 mo



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Proof of concept

- Significantly lower FEC over sampling frame: mean reduction 70% (1) & 58% (2)
  - At peak, vaccinates shed 92% (1) & 73% (2) fewer eggs than controls
  - Mean reductions of 75% (1) & 56% (2) in adult nematodes
- Protection variable in lambs < 5 mo

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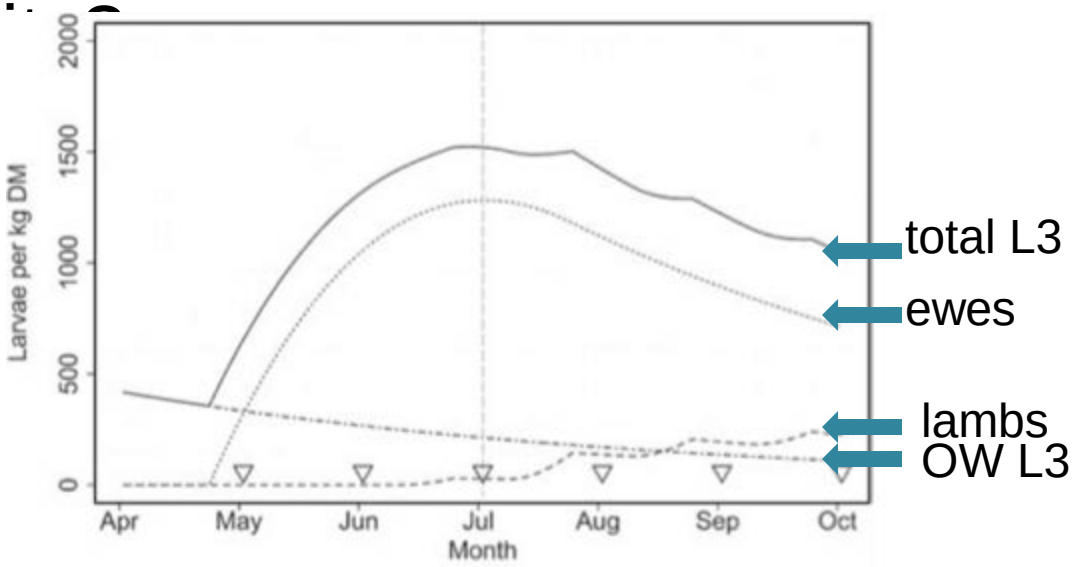
PPRI

Benchmark Animal Health

- Reduction in immunity to new & current infections
- Increase in FEC acts as source of contamination for lambs for rest of season
- Can vaccination overcome relaxation in immun



Singleton et al. 2011 Parasitol. 138:322-32.

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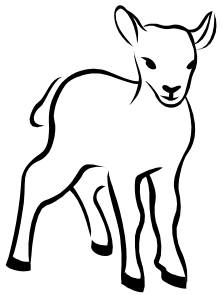
Working hypothesis

integrated control with targeted drug treatments

ewe PPRI

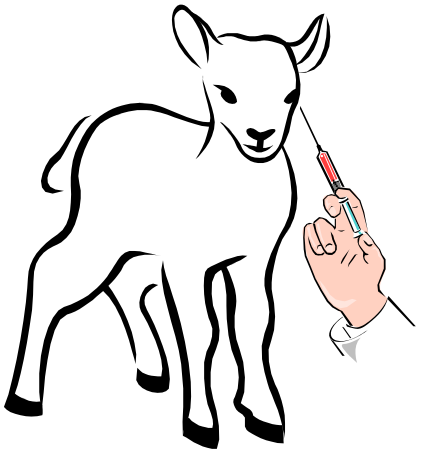
reduce contamination early in season

young lambs



can we improve effect in young lambs?

older lambs



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Conclusions & further work

- Protection higher than observed in any system using recombinant vaccine *vs*. a nematode in the ruminant host
  - Simplify the cocktail
- Investigate mechanisms behind variation in responsiveness
- Investigate effect on PPRI
  - one experiment per year!

F. hepatica

- Native preparations give good efficacy
- Recombinant proteins have shown variable efficacy
- Most promising: leucine aminopeptidase

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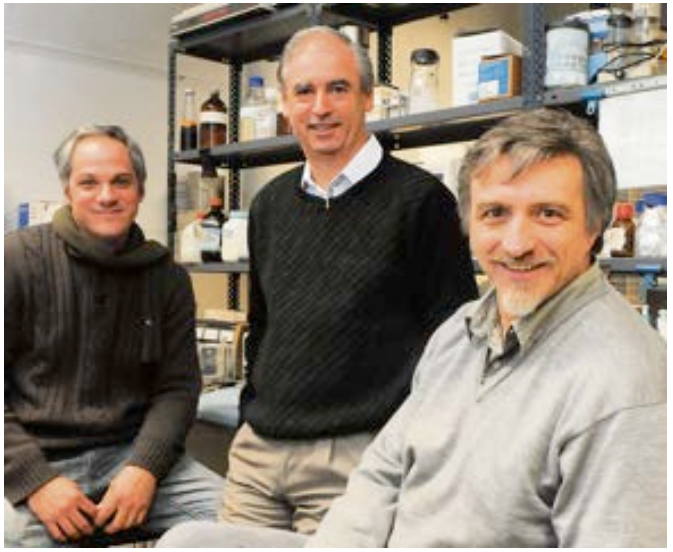
Fasciola LAP

- Recombinant LAP in *E. coli*
  - 2 immunisations; 100 µg LAP with Freund's complete plus incomplete adjuvant, Alum, Adyuvac 50, DEAE-D Ribi
  - 2 weeks after booster, oral challenge with 200 metacercariae
- Significant reduction in fluke burdens in all groups
  - Highest = 86.7% (Alum)



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PARAGONE

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Partnership: academics, pharma, SMEs

- To develop at least two multicellular parasite vaccines towards commercialisation
- Target hosts: cattle, sheep, poultry
- Ideally, recombinant vaccines

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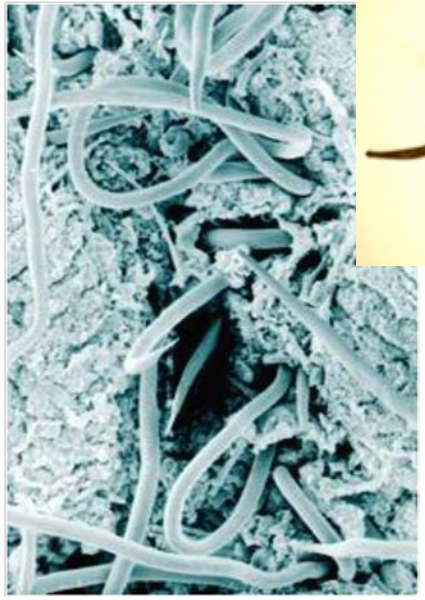

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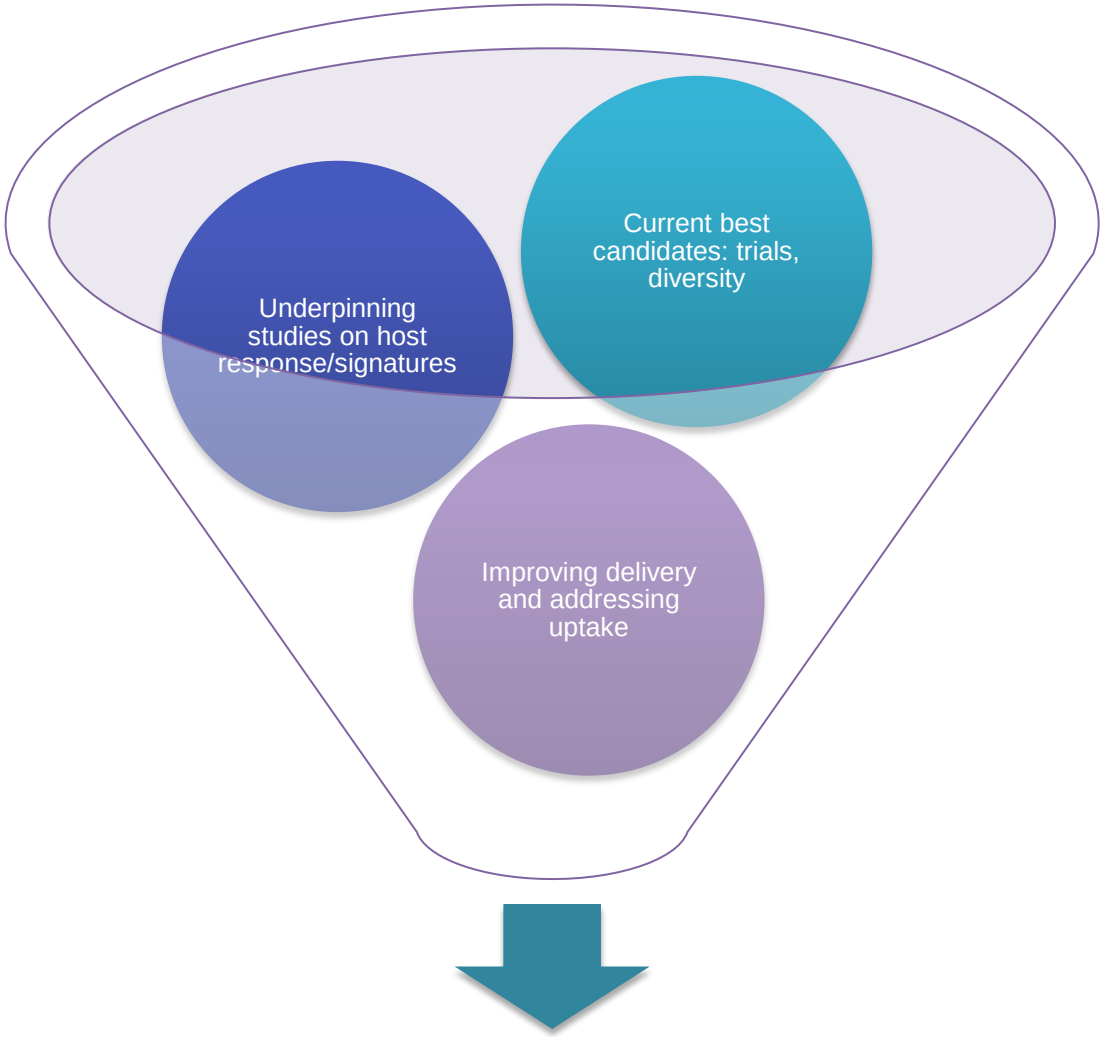
Ethos

“A successful anti-nematode vaccine is likely to be a multi-component vaccine involving antigens expressed by different developmental stages of the parasite”, Peter Hotez

Strategy



Commercially viable anti-parasite vaccines

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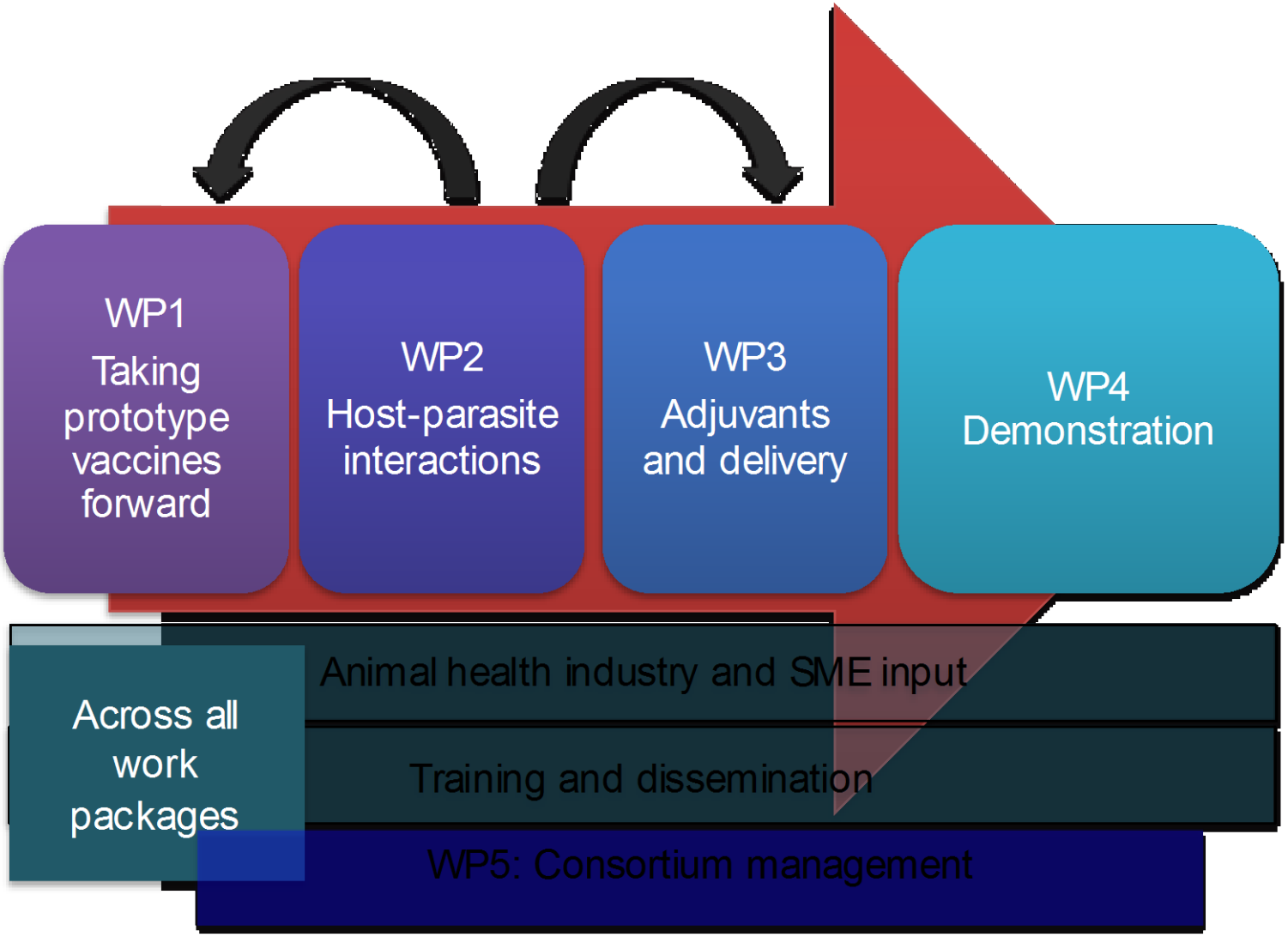

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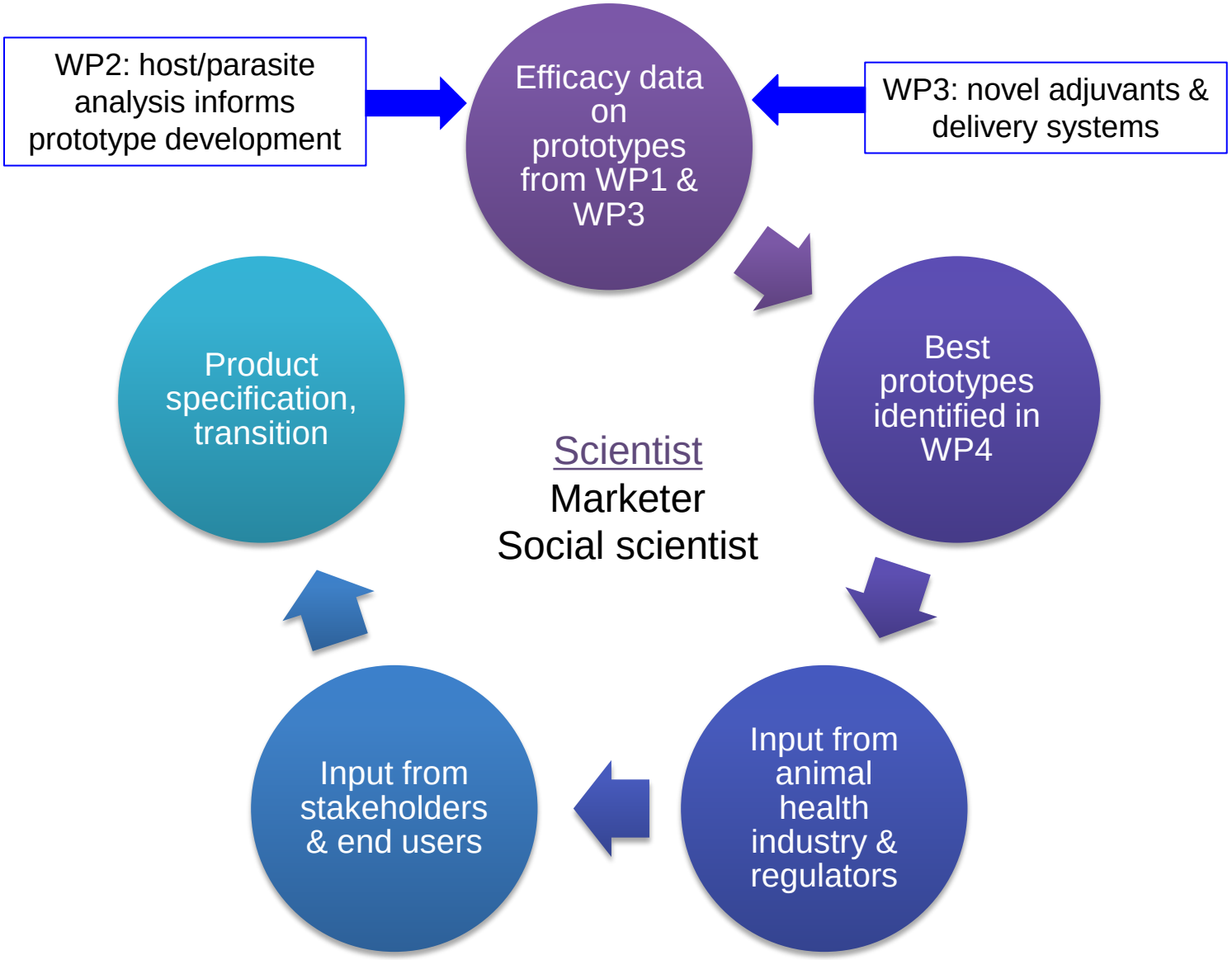
Innovation to exploitation



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