Development of a new subcellular vaccine to protect sheep from ovine enzootic abortion

Longbottom, D[1]; Livingstone, M[1]; Wattegedera, S[1]; Palarea, J[2]; Aitchison, K[1]; Chianini, F[1]; Rocchi, M[1]; Wheelhouse, N[1]; Entrican, G[1].

[1] **Moredun Research Institute, Pentlands Science Park, Edinburgh, UK**
[2] **Biomathematics & Statistics Scotland, Invergowrie, Dundee, UK**

The Scottish Government
Riaghaltas na h-Alba

ESCCAR / 9th ESCR, Lausanne, Switzerland, 24AUG22

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Ovine Enzootic Abortion (OEA)

- aka chlamydial abortion, ovine chlamydiosis & Enzootic Abortion of Ewes (EAE)
- Late term abortion/stillborn lamb 2-3 weeks prior to lambing
- Little or no prior warning
- Vaginal discharge – infectious EBs
- Placenta – necrotic, inflammed, oedematous – thickened, dirty 'pinkish-straw coloured' exudate
- Ewe can produce affected (dead/weak) and normal healthy lambs
- Ewes can continue to excrete organisms in vulval discharge for several days

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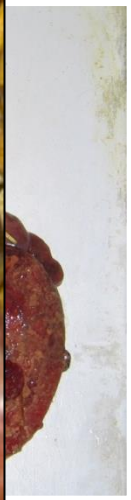

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Chlamydia abortus

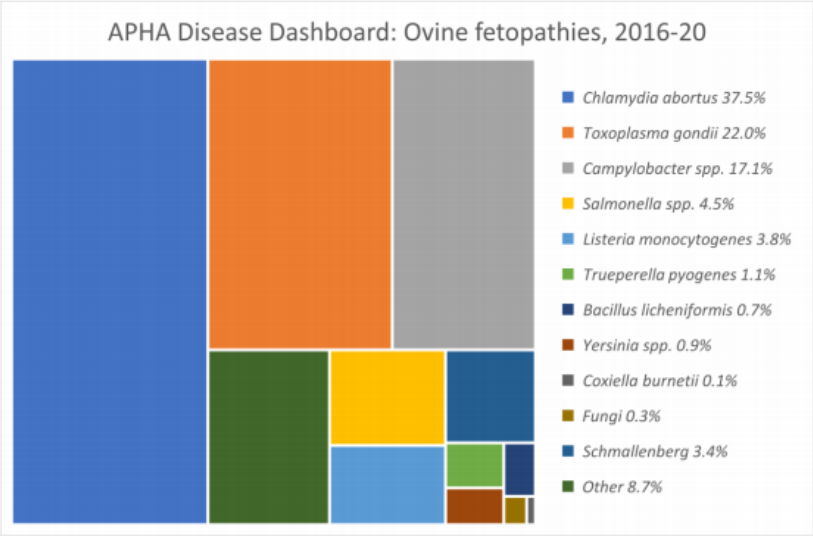
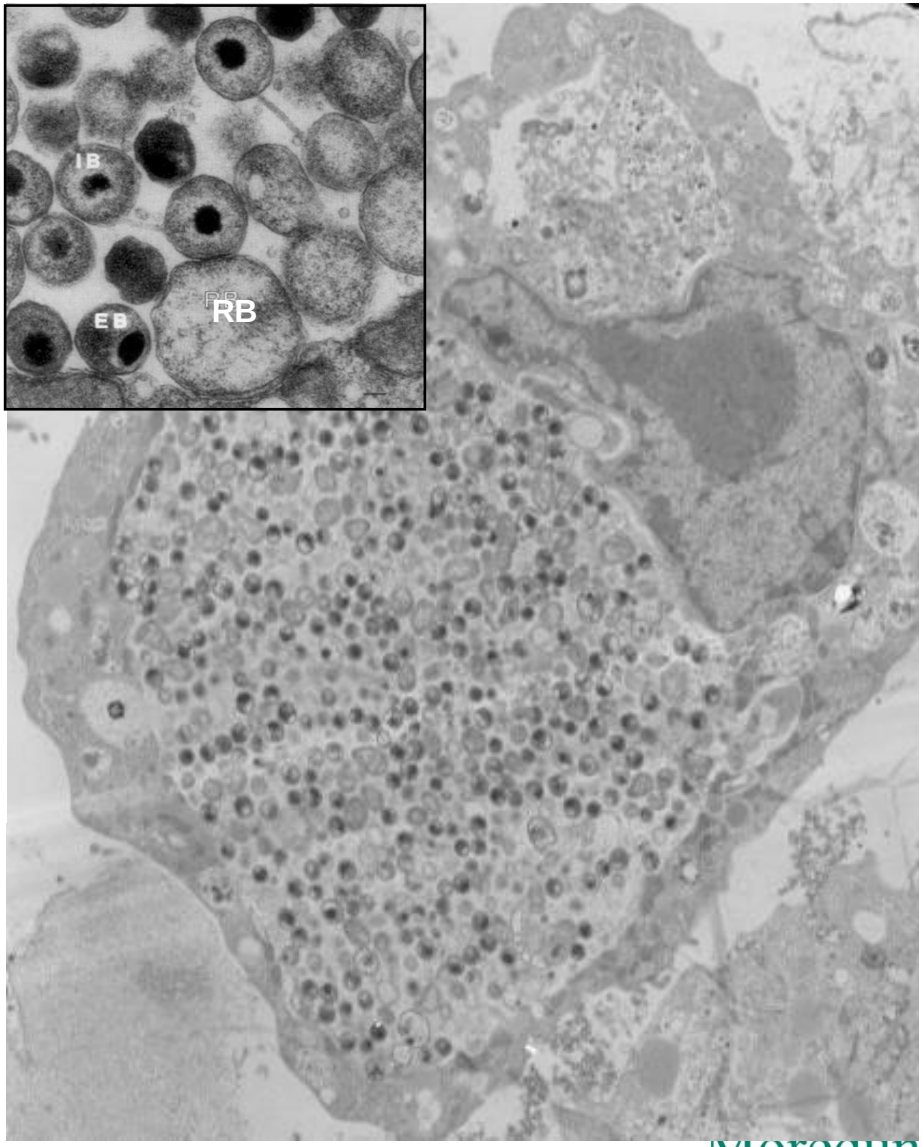


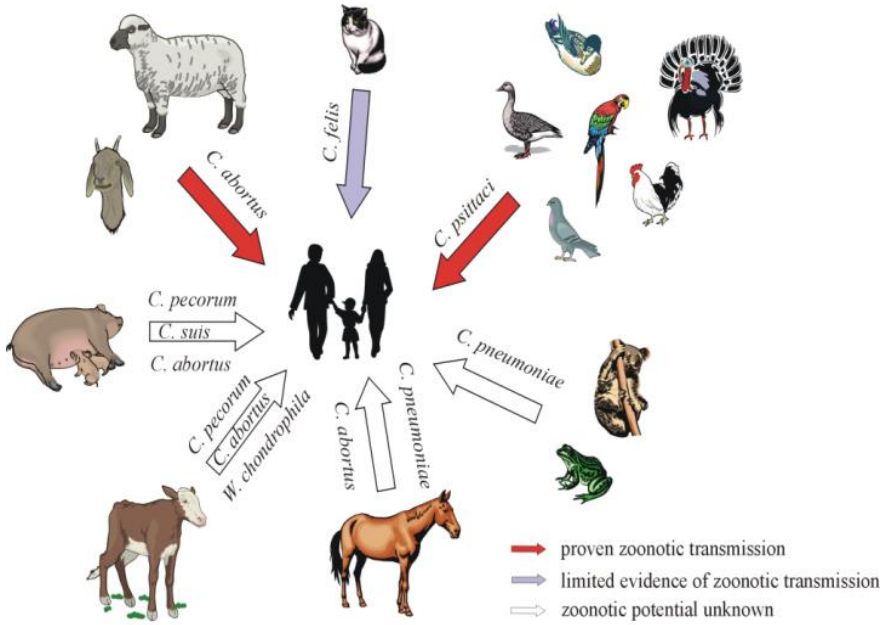
Figure 1.2 UK incidence of diagnosed ovine fetopathies

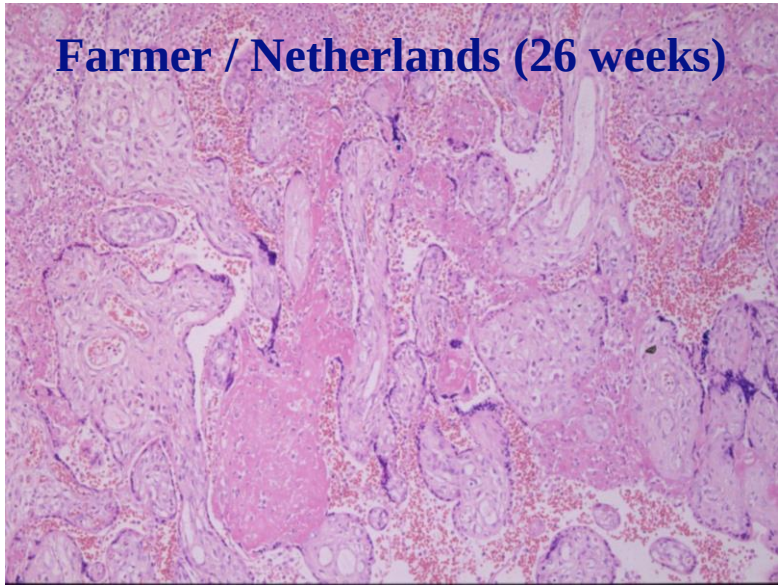
The treemap provides an overview the diagnosed ovine fetopathies in the UK. The data presented as percentages of combined datasets of disease incidence between 2016 and 2020. The data was complied from the UK government veterinary disease surveillance network, where it was published by the Animal and Plant Health Agency online, last updated 04 June 2020, last accessed website 04 May 2021 (Animal and Plant Heath Agency, 2021)



Zoonotic

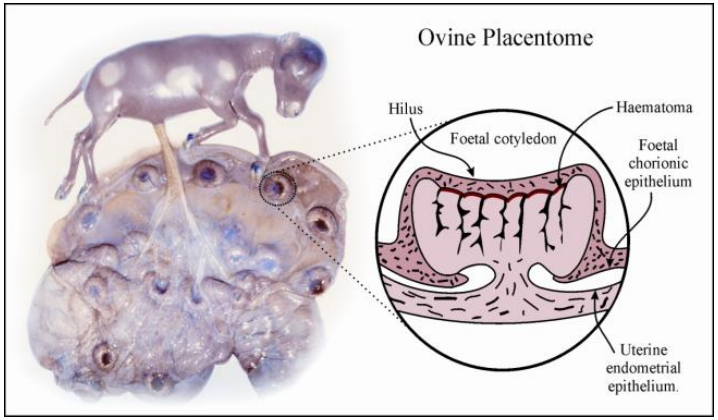
- Fever , headache, nausea, malaise
- Risk of spontaneous abortion in pregnant women
- Can be life-threatening for pregnant woman
  - disseminated intravascular coagulation (DIC)
  - profound thrombocytopenia
  - renal/hepatic dysfunction
  - myocardial symptoms





Disease characteristics

- Following infection organism becomes undetectable in non-pregnant ewe
  - latency involves suppression of the organism by interferon-γ
- Infection in female only becomes apparent during pregnancy
  - organism detectable at 80-95 dg
- Infection establishes in chorionic epithelium (trophoblast)
  - necrosis develops in placenta
  - abortion occurs at 125-140 dg
- After abortion maternal antibody levels rise which coincides with the ewe developing protective immunity



- Protective immunity involves both humoral and cell-mediated responses
  - Cell mediated response more important in a primary infection
  - CD4+ Th1 IFN-γ response

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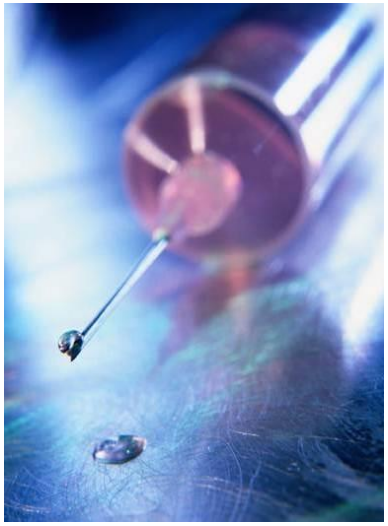
Disease Control

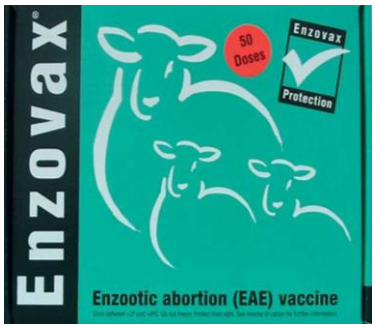
- Management, treatment and diagnosis
- Diagnosis: confirmative
- Antibiotics: oxytetracycline (95-105 dg)
  - *Limit losses*
  - *Will not prevent established disease*
- Vaccination: best option long term, existing infections

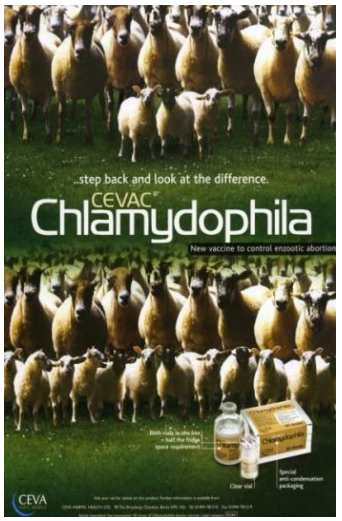


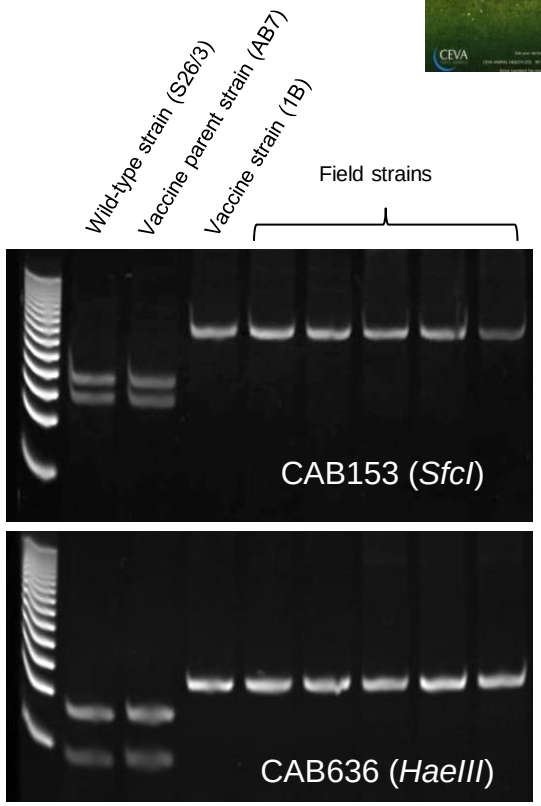
Vaccination

- Two types of vaccines available commercially:
  - Two live, attenuated (ts mutant) vaccines
  - Inactivated vaccine (INMEVA; Salmonella abortus ovis; Montbrau et al., 2020)

- Issues with vaccines:
  - Production costs (growth in eggs)
  - Stability (shelf-life and following reconstitution, requiring cold-chain)
  - Efficacy and duration of immunity (inactivated vaccines)
  - Safety (live vaccines)
    - Humans – zoonotic
    - Animal – demonstrated to cause disease (molecular DIVA (PCR/RFLP))







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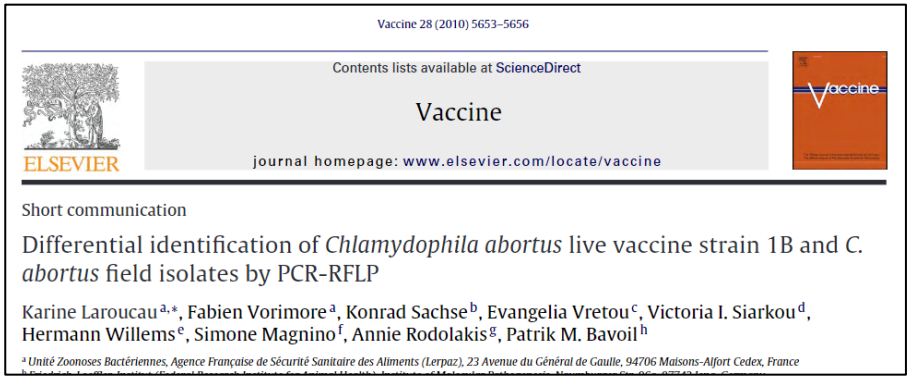


Vaccine 28 (2010) 5653–5656

Contents lists available at ScienceDirect

Vaccine

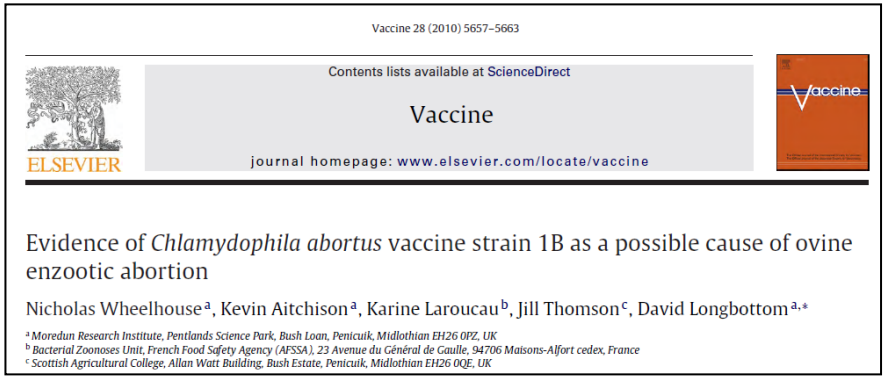
journal homepage: www.elsevier.com/locate/vaccine

Short communication

Differential identification of *Chlamydophila abortus* live vaccine strain 1B and *C. abortus* field isolates by PCR-RFLP

Karine Laroucau [a,*], Fabien Vorimore [a], Konrad Sachse [b], Evangelia Vretou [c], Victoria I. Siarkou [d], Hermann Willems [e], Simone Magnino [f], Annie Rodolakis [g], Patrik M. Bavoil [h]

[a] Unité Zoonoses Bactériennes, Agence Française de Sécurité Sanitaire des Aliments (Lerpaz), 23 Avenue du Général de Gaulle, 94706 Maisons-Alfort Cedex, France



Vaccine 28 (2010) 5657–5663

Contents lists available at ScienceDirect

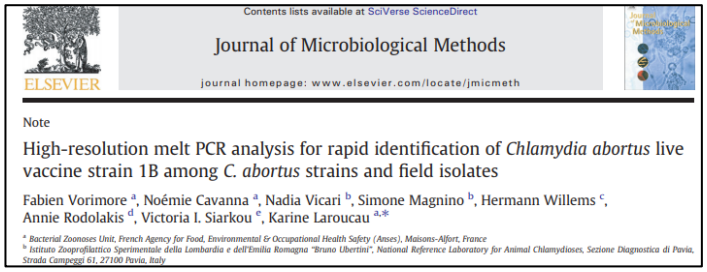
Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Evidence of *Chlamydophila abortus* vaccine strain 1B as a possible cause of ovine enzootic abortion

Nicholas Wheelhouse [a], Kevin Aitchison [a], Karine Laroucau [b], Jill Thomson [c], David Longbottom [a,*]

[a] Moredun Research Institute, Pentlands Science Park, Bush Loan, Penicuik, Midlothian EH26 0PZ, UK
[b] Bacterial Zoonoses Unit, French Food Safety Agency (AFSSA), 23 Avenue du Général de Gaulle, 94706 Maisons-Alfort cedex, France
[c] Scottish Agricultural College, Allan Watt Building, Bush Estate, Penicuik, Midlothian EH26 0QE, UK



Contents lists available at SciVerse ScienceDirect

Journal of Microbiological Methods

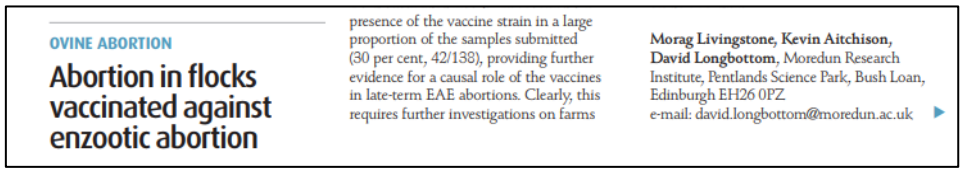
journal homepage: www.elsevier.com/locate/jmicmeth

Note

High-resolution melt PCR analysis for rapid identification of *Chlamydia abortus* live vaccine strain 1B among *C. abortus* strains and field isolates

Fabien Vorimore [a], Noémie Cavanna [a], Nadia Vicari [b], Simone Magnino [b], Hermann Willems [c], Annie Rodolakis [d], Victoria I. Siarkou [e], Karine Laroucau [a,*]

[a] Bacterial Zoonoses Unit, French Agency for Food, Environmental & Occupational Health Safety (Anses), Maisons-Alfort, France
[b] Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna "Bruno Ubertini", National Reference Laboratory for Animal Chlamydioses, Sezione Diagnostica di Pavia, Strada Campeggi 61, 27100 Pavia, Italy

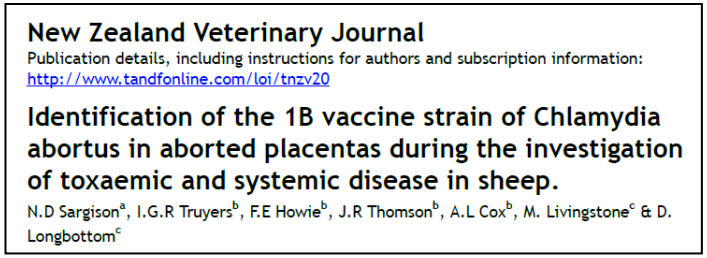


OVINE ABORTION

Abortion in flocks vaccinated against enzootic abortion

presence of the vaccine strain in a large proportion of the samples submitted (30 per cent, 42/138), providing further evidence for a causal role of the vaccines in late-term EAE abortions. Clearly, this requires further investigations on farms

Morag Livingstone, Kevin Aitchison, David Longbottom, Moredun Research Institute, Pentlands Science Park, Bush Loan, Edinburgh EH26 0PZ
e-mail: david.longbottom@moredun.ac.uk

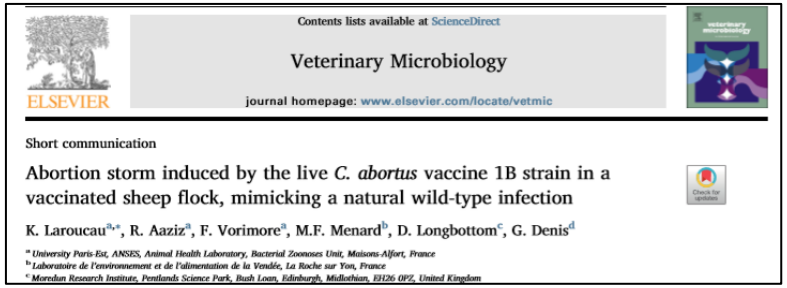


New Zealand Veterinary Journal

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/tnzv20

Identification of the 1B vaccine strain of Chlamydia abortus in aborted placentas during the investigation of toxaemic and systemic disease in sheep.

N.D Sargison[a], I.G.R Truyers[b], F.E Howie[b], J.R Thomson[b], A.L Cox[b], M. Livingstone[c] & D. Longbottom[c]



Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Short communication

Abortion storm induced by the live *C. abortus* vaccine 1B strain in a vaccinated sheep flock, mimicking a natural wild-type infection

K. Laroucau[a,*], R. Aaziz[a], F. Vorimore[a], M.F. Menard[b], D. Longbottom[c], G. Denis[d]

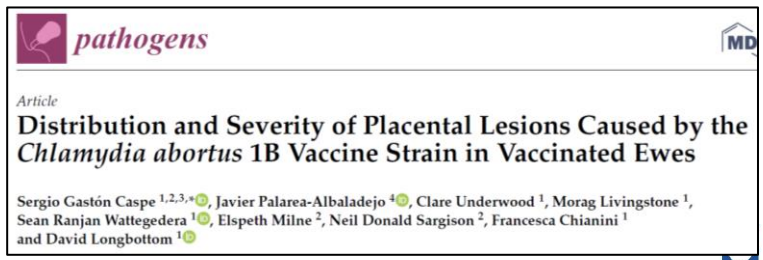
[a] University Paris-Est, ANSES, Animal Health Laboratory, Bacterial Zoonoses Unit, Maisons-Alfort, France
[b] Laboratoire de l'environnement et de l'alimentation de la Vendée, La Roche sur Yon, France
[c] Moredun Research Institute, Pentlands Science Park, Bush Loan, Edinburgh, Midlothian, EH26 0PZ, United Kingdom

PLOS ONE

RESEARCH ARTICLE

The 1B vaccine strain of *Chlamydia abortus* produces placental pathology indistinguishable from a wild type infection

Sergio Gaston Caspe[1,2,3*], Morag Livingstone[1], David Frew[1], Kevin Aitchison[1], Sean Ranjan Wattegedera[1], Gary Entrican[1], Javier Palarea-Albaladejo[4], Tom Nathan McNeilly[1], Elspeth Milne[2], Neil Donald Sargison[2], Francesca Chianini[1], David Longbottom[1]



pathogens

Article

Distribution and Severity of Placental Lesions Caused by the *Chlamydia abortus* 1B Vaccine Strain in Vaccinated Ewes

Sergio Gastón Caspe [1,2,3,*], Javier Palarea-Albaladejo [4], Clare Underwood [1], Morag Livingstone [1], Sean Ranjan Wattegedera [1], Elspeth Milne [2], Neil Donald Sargison [2], Francesca Chianini [1] and David Longbottom [1]

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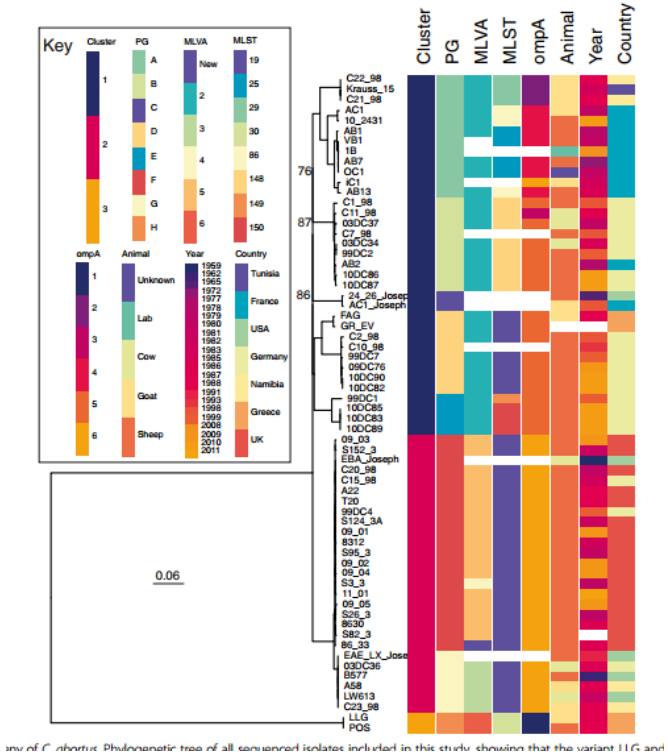
Whole genome sequencing

WGS & comparative analysis – vaccine strain (1B), reverted mutant strain (1H) & vaccine parent strain (AB7)



Vaccine 36 (2018) 3593–3598

Contents lists available at ScienceDirect

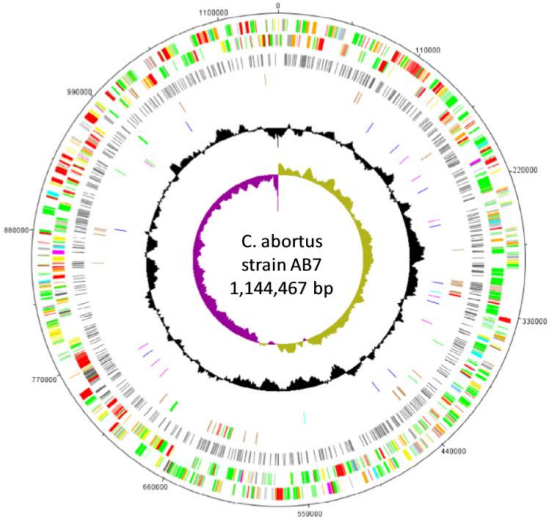
Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Genomic evidence that the live *Chlamydia abortus* vaccine strain 1B is not attenuated and has the potential to cause disease

David Longbottom [a,*], Michelle Sait [a,1], Morag Livingstone [a], Karine Laroucau [b], Konrad Sachse [c,2], Simon R. Harris [d], Nicholas R. Thomson [d], Helena M.B. Seth-Smith [d,3]

[a] Moredun Research Institute, Pentlands Science Park, Bush Loan, Edinburgh, Midlothian EH26 0PZ, United Kingdom
[b] Bacterial Zoonoses Unit, French Agency for Food, Environmental & Occupational Health Safety (Anses), Maisons-Alfort, France
[c] Friedrich-Loeffler-Institute (Federal Research Institute for Animal Health), Institute of Molecular Pathogenesis, Jena, Germany
[d] Infection Genomics, Wellcome Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridgeshire CB10 1SA, United Kingdom



Evidence of the need for a new safer vaccine

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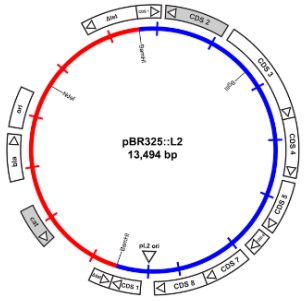
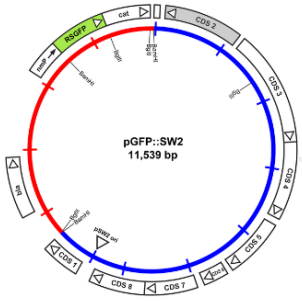
Vaccine development - approaches

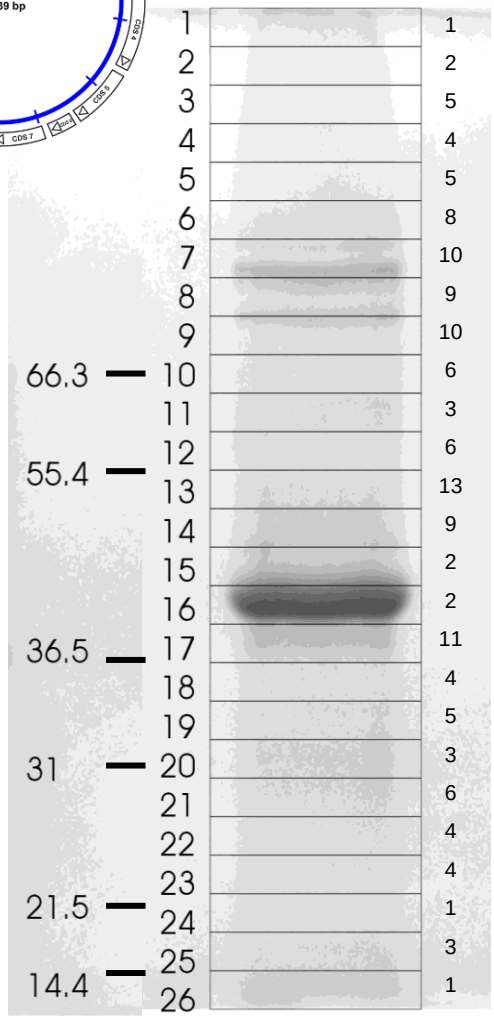
- Mutant attenuated strain:
  - Transformation
  - Gene knockout studies





- Recombinant antigens:
  - Candidate antigens
  - Expression, solubility & delivery
  - Renaturation studies
  - Elicit required protective immune responses

- Subunit preparations:
  - Chlamydial outer membrane preparation (COMC)
  - OG-COMC



Pregnant ewe vaccine trials

- **Trial 1** - antigen comparisons (commercial live vaccine, subunit vaccines)
- **Trial 2** - vaccine regimen (1 dose or 2 doses)
- **Trial 3** – dose titration (20µg → 2.5µg)
- **Trial 4** – adjuvant comparisons (ISA70VG, ISA61VG, QuilA)

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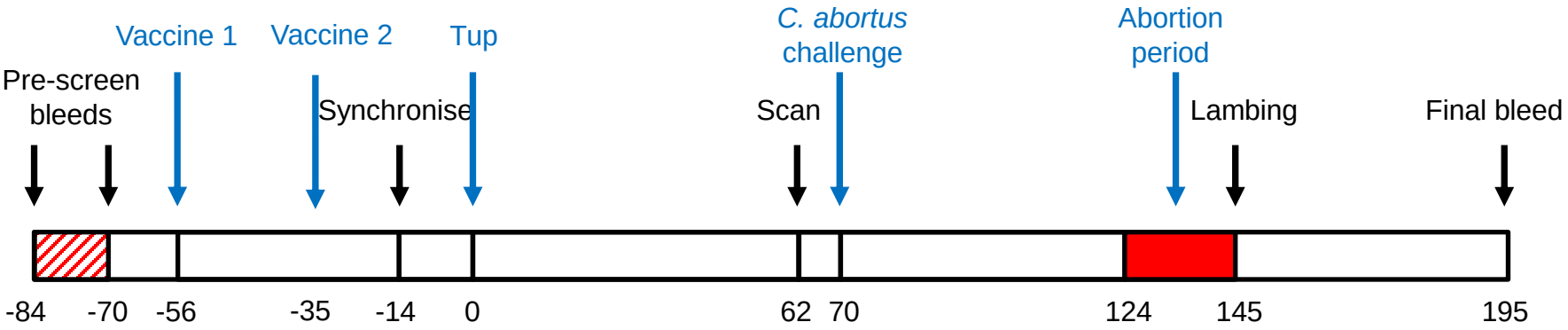
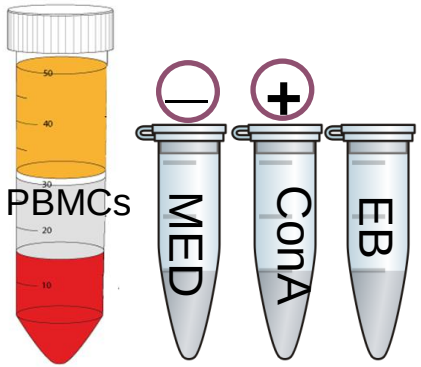
Vaccine trial 1



Group	Ewes	Treatment
1	25	Commercial live vaccine
2	26	2 x COMC 10 µg/ISA70VG
3	24	2 x OG-COMC 10 µg/ISA70VG
4	25	Challenge control
5	25	Negative control

150 ewes pre-screened
125 selected for study



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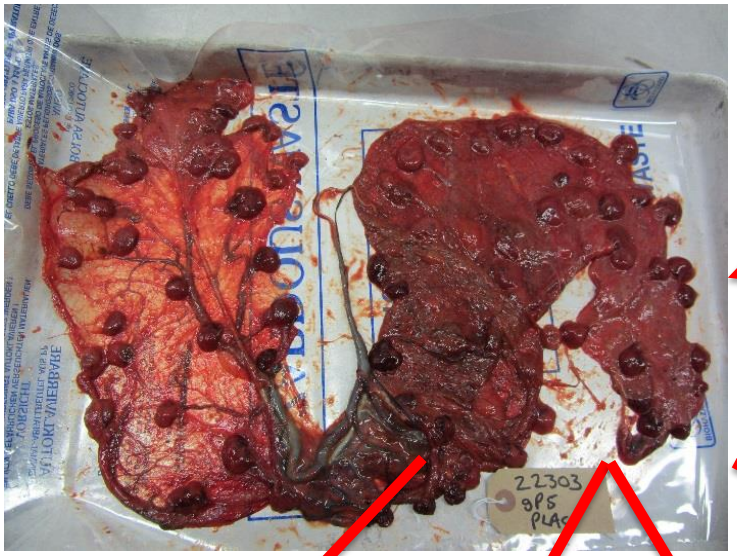
Vaccine trials

Placenta

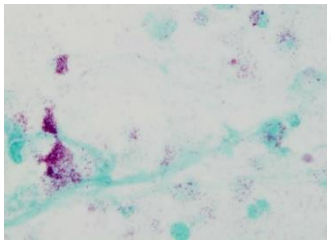


Gross pathology assessment

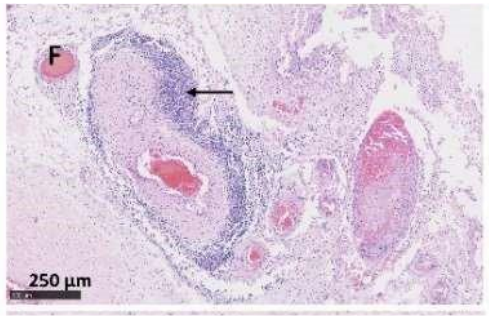
Confirmation: qPCR

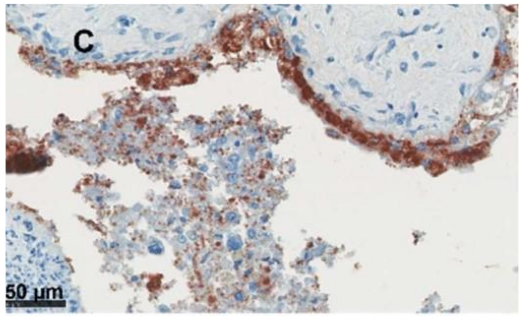
Shedding (pathogen detection): vaginal swab (qPCR)

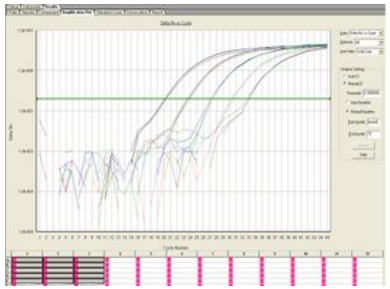
Pathogen detection: Placental smear (mZN)



Confirmation: histology & IHC







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Vaccine trial 1

- 2 vaccine formulations compared to commercial vaccine
- Adjuvant - Montanide ISA70VG (SEPPIC)
- 2 doses of 10µg protein each (im)
- Challenge 70 dg (sc)

vaccines

MDPI

Article

Efficacy of Two *Chlamydia abortus* Subcellular Vaccines in a Pregnant Ewe Challenge Model for Ovine Enzootic Abortion

Morag Livingstone [1], Sean Ranjan Wattegedera [1], Javier Palarea-Albaladejo [2,†], Kevin Aitchison [1], Cecilia Corbett [1,‡], Michelle Sait [1,§], Kim Wilson [1,‖], Francesca Chianini [1], Mara Silvia Rocchi [1], Nicholas Wheelhouse [1,¶], Gary Entrican [1,*] and David Longbottom [1,*]

Group	Ewes	Treatment
1	25	Commercial live vaccine
2	26	2 x COMC 10 µg/ISA70VG
3	24	2 x OG-COMC 10 µg/ISA70VG
4	25	Challenge control
5	25	Negative control

Table 1. The clinical outcome of pregnancy in vaccinated ewes that were subsequently challenged with *Chlamydia abortus* at day 70 of gestation (groups 1–3), in infected control ewes (group 4), or in uninfected control ewes (group 5).

Group	Ewes			Number of Lambs			
	No. Pregnant	No. Lambed (%)	No. Aborted (%)	Mean Gestational Length	Viable	Non-Viable [1]	Dead
1	25	24 (96)	1 (4)	142	41	0	2 [2]
2	26	26 (100)	0 (0)	144	47	0	0
3	24	24 (100)	0 (0)	144	39	0	0
4	25	12 (48)	13 (52)	135	23	5	20
5	25	25 (100)	0 (0)	144	43	0	0

[1] includes neonatal deaths (born live but died within 48 h) and stillbirths. [2] includes death of one lamb due to dystocia/asphyxiation.

Table 2. Gross placental pathology, detection of *Chlamydia abortus* organisms in placental smears and detection of genomic DNA in vaginal swabs of vaccinated ewes that were challenged with *C. abortus* at day 70 of gestation (groups 1–3), of infected control ewes (group 4), and of uninfected control ewes (group 5).

Group	Pregnancy Outcome [1]	No. Ewes	Lesions [2]	mZN [3]	Placental qPCR [4]	Swab qPCR [5]
1	Lambed	24 [6]	3+, 21−	3+, 21−	9+, 15−	90.7 (1.65)
	Aborted	1	1+	1+	1+	- [7]
2	Lambed	26	0+, 26−	3+, 23−	6+, 20−	49.13 (1.71)
3	Lambed	24	3+, 21−	3+, 21−	14+, 10−	73.63 (2.2)
4	Lambed	12	11+, 1−	11+, 1−	12+, 0−	43651.42 (3.39)
	Aborted	13	13+, 0−	13+, 0−	13+, 0−	919232.3 (1.78)
5	Lambed	25	25−	25−	25−	12.14 (1.19)

[1] See Table 1. [2] Number of ewes with gross pathological lesions characteristic of *C. abortus* infection evident in one or more placentas. [3] Detection of chlamydial organisms following staining of placental smears: +, positive; −, negative. [4] Quantitative real-time polymerase chain reaction (qPCR) detection of *C. abortus* DNA in the cotyledons tested by modified Ziehl-Neelsen (mZN). [5] Geometric mean (geometric SEM) of the number of *C. abortus* genomes detected per µL total DNA of swab extract. [6] Includes lamb that died of dystocia/asphyxiation. [7] The summary statistics cannot be computed for a single value.

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Vaccine trial 1

- 3 vaccine formulations compared to commercial vaccine
- Adjuvant - Montanide ISA70VG (SEPPIC)
- 2 doses of 10µg protein each (im)
- Challenge 70 dg (sc)

vaccines

MDPI

Article

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Morag Livingstone [1], Sean Ranjan Wattegedera [1], Javier Palarea-Albaladejo [2,†], Kevin Aitchison [1], Cecilia Corbett [1,‡], Michelle Sait [1,§], Kim Wilson [1,‖], Francesca Chianini [1], Mara Silvia Rocchi [1], Nicholas Wheelhouse [1,⊥], Gary Entrican [1,*] and David Longbottom [1,*]

Group	Ewes	Treatment
1	25	Commercial live vaccine
2	26	2 x COMC 10 µg/ISA70VG
3	24	2 x OG-COMC 10 µg/ISA70VG
4	25	Challenge control
5	25	Negative control

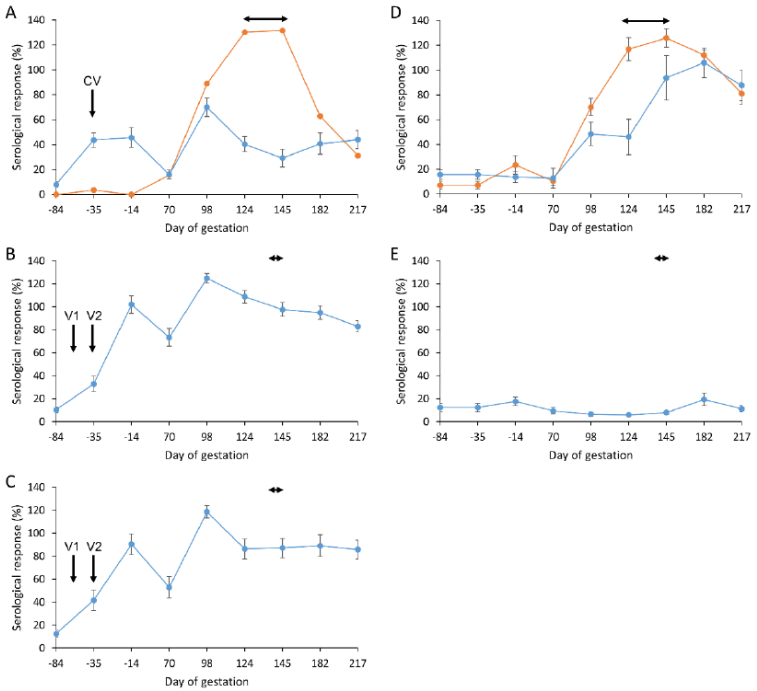

Figure 2. Serological responses following vaccination and challenge with *Chlamydia abortus*. Detection of *C. abortus* antibody in ewes vaccinated (V1 and CV/V2—see Figure 1) with commercial vaccine 1 (**A**) or experimental vaccines 2 (**B**) and 3 (**C**) and challenged at day 70 of gestation with *C. abortus* strain S26/3. Unvaccinated challenged (**D**) and unvaccinated non-challenged (**E**) ewes served as positive and negative control groups. Data are separated into lambed (blue lines) versus aborted (orange lines). Data points represent the arithmetic mean values for each cellular bleed and error bars represent the standard error of that mean (SEM). The 100% is equivalent to an OD450 nm of 2.25. The lambing/abortion period is indicated by the horizontal double-headed arrows.

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Vaccine trial 1

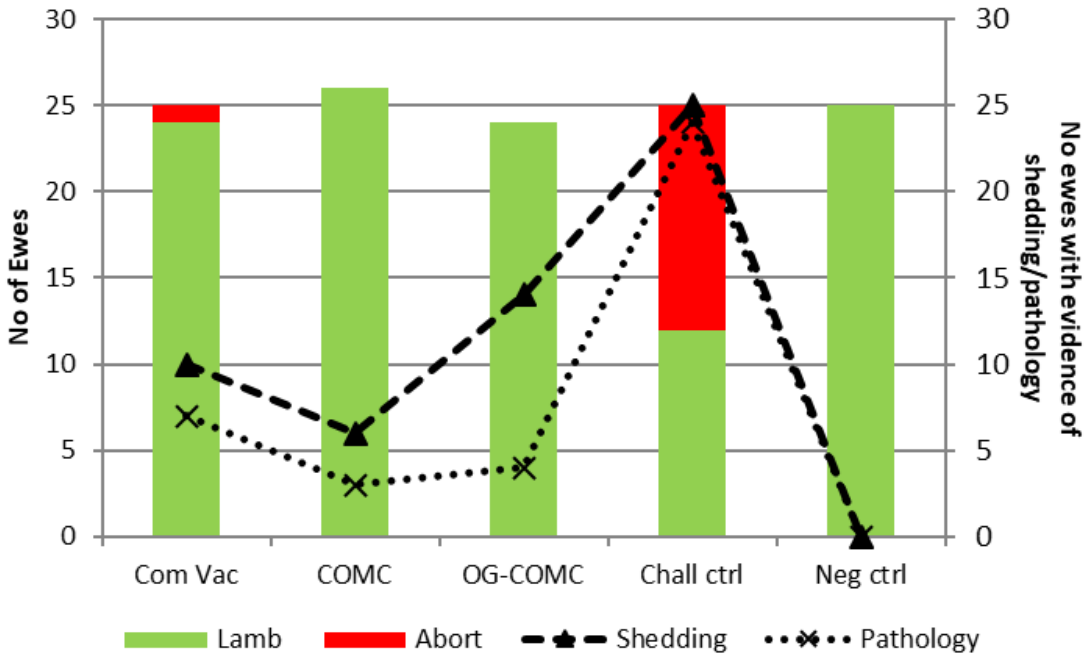
- 3 vaccine formulations compared to commercial vaccine
- Adjuvant - Montanide ISA70VG (SEPPIC)
- 2 doses of 10µg protein each (im)
- Challenge 70 dg (sc)
- *No abortions in Vac1 (COMC) or Vac2 (OG-COMC)*
- *Lowest shedding and pathology in Vac1*

vaccines MDPI

Article

Efficacy of Two *Chlamydia abortus* Subcellular Vaccines in a Pregnant Ewe Challenge Model for Ovine Enzootic Abortion

Morag Livingstone [1], Sean Ranjan Wattegedera [1], Javier Palarea-Albaladejo [2,†], Kevin Aitchison [1], Cecilia Corbett [1,‡], Michelle Sait [1,§], Kim Wilson [1,‖], Francesca Chianini [1], Mara Silvia Rocchi [1], Nicholas Wheelhouse [1,¶], Gary Entrican [1,*] and David Longbottom [1,*]



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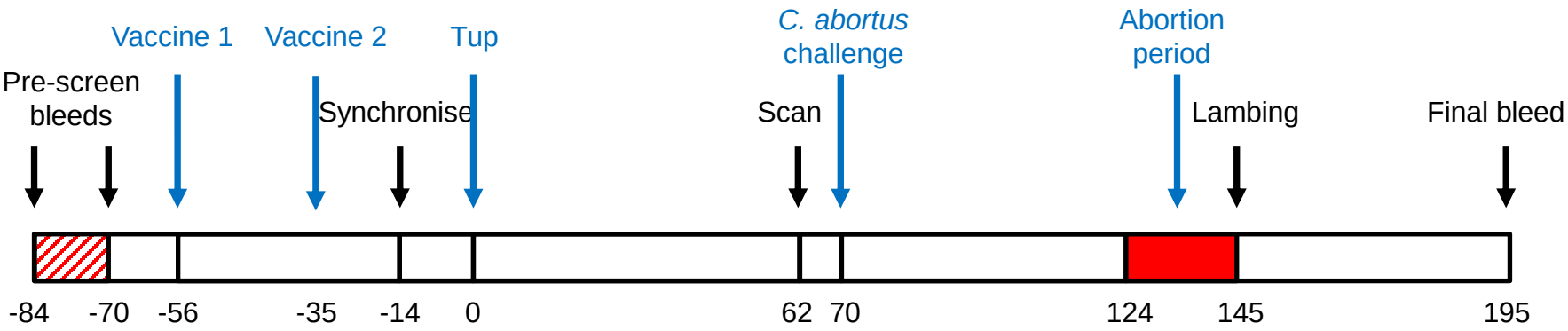
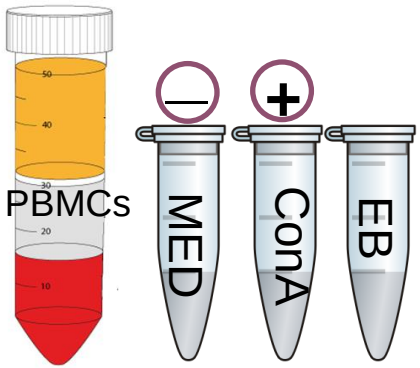
Vaccine trial 2



Group	Ewes	Treatment
1	24	2 x 10 µg COMC/ISA70VG
2	25	1 x 20 µg COMC/ISA70VG
3	23	2 x 5 µg COMC/ISA70VG
4	22	1 x 10 µg COMC/ISA70VG
5	25	Challenge control
6	23	Negative control

190 ewes pre-screened
162 selected for study



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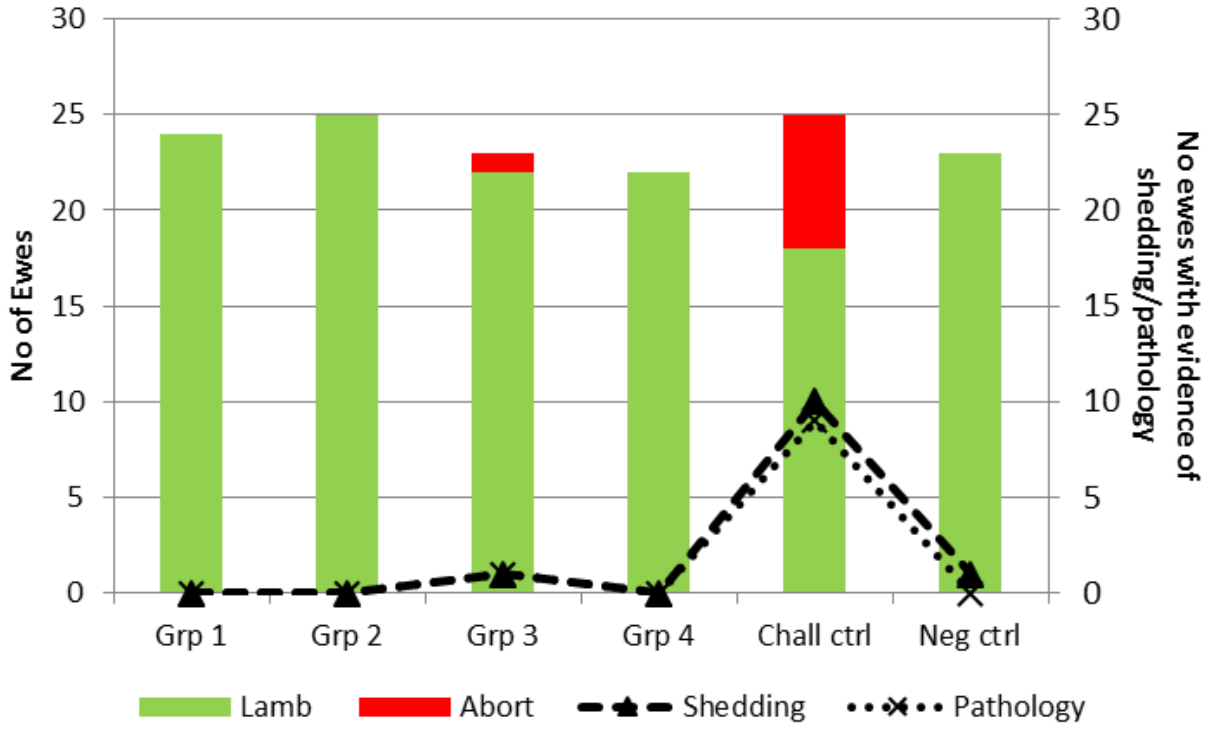
Vaccine trial 2

- COMC Ag
- Single dose versus double dose
- Grp 1 (2x10µg), Grp 2 (1x20µg), Grp 3 (2x5µg), Grp 4 (1x10µg), Grp 5 (Chall ctrl), Grp 6 (Neg ctrl)
- *Single inoculation OK*
- *Lower dilution of Ag possible?*



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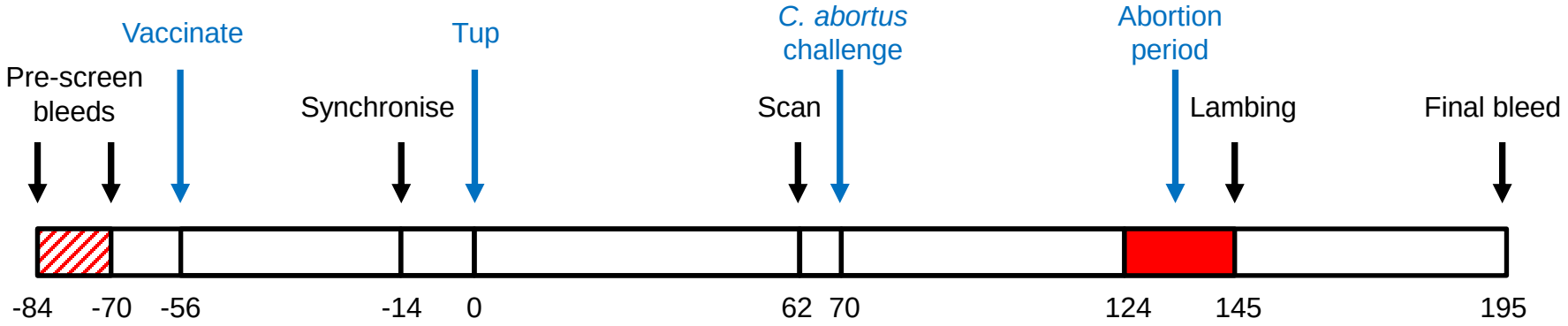
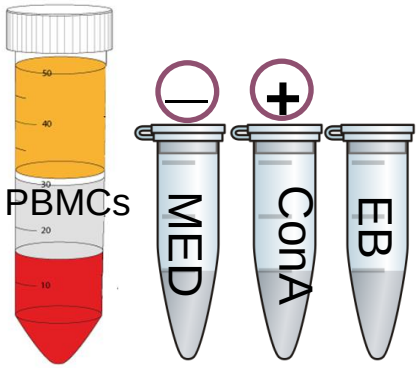
Vaccine trial 3



Group	Ewes	Treatment
1	19	20 µg COMC/ISA70VG
2	19	15 µg COMC/ISA70VG
3	21	10 µg COMC/ISA70VG
4	20	7.5 µg COMC/ISA70VG
5	18	5 µg COMC/ISA70VG
6	18	3.5 µg COMC/ISA70VG
7	21	2.5 µg COMC/ISA70VG
8	19	Challenge control
9	6	Negative control

175 ewes pre-screened
151 selected for study



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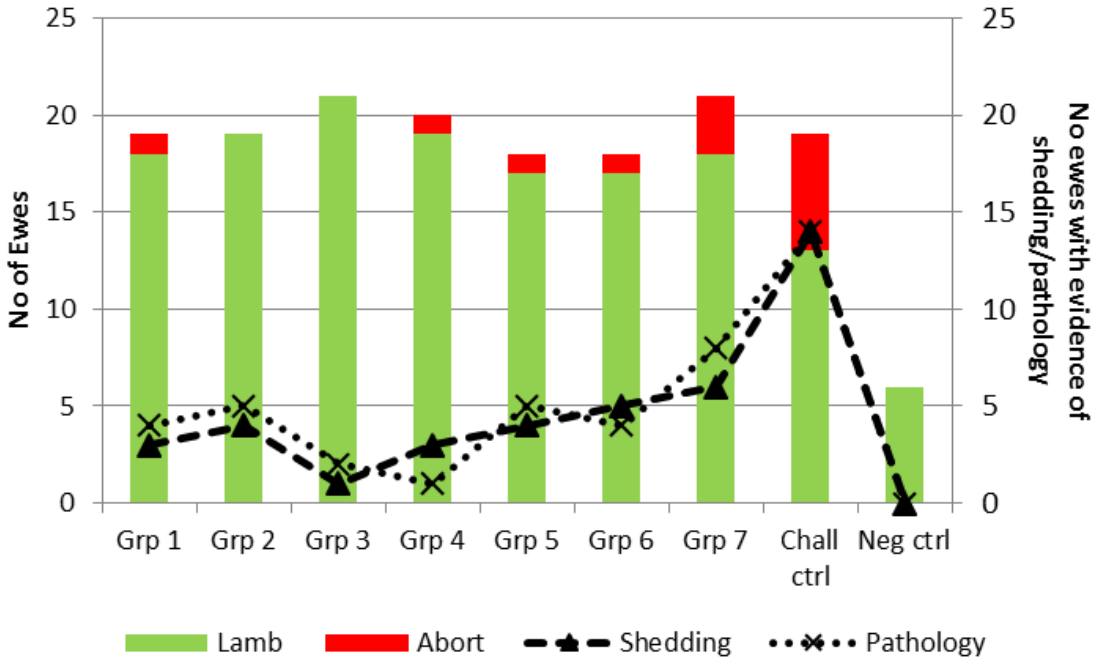
Vaccine trial 3

- Single dose of COMC Ag
- Ag dose response
- Grp 1 (20µg), Grp 2 (15µg), Grp 3 (10µg), Grp 4 (7.5µg), Grp 5 (5µg), Grp 6 (3.5µg), Grp 7 (2.5µg), Grp 8 (Chall ctrl), Grp 9 (Neg ctrl)
- *No obvious dose response*
- *Lowest dose most abortions*
- *Adjuvant?*



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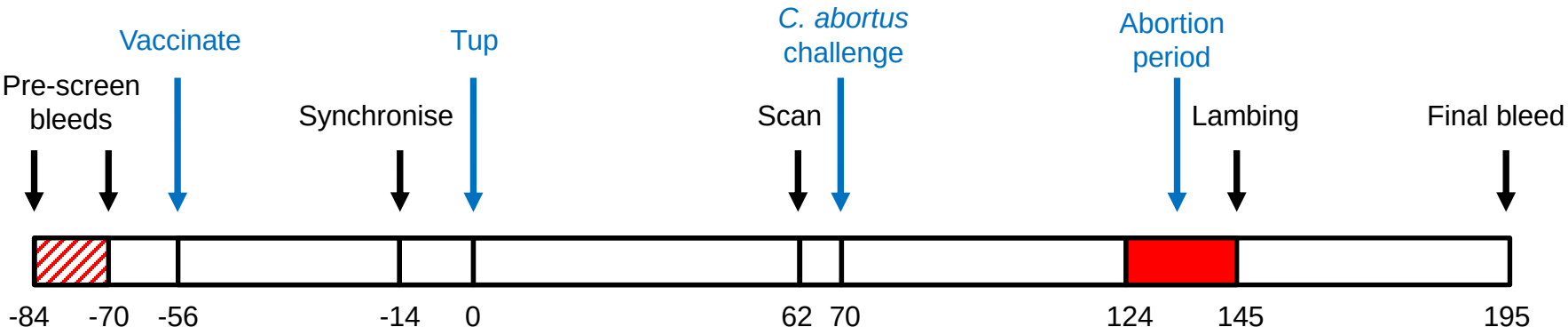
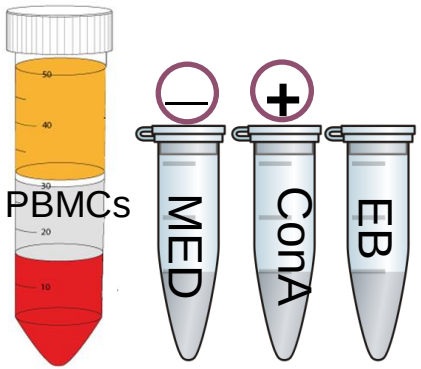
Vaccine trial 4



Group	Ewes	Treatment
1	37	20 µg COMC/ISA70VG
2	32	2.5 µg COMC/ISA70VG
3	37	2.5 µg COMC/ISA61VG
4	38	2.5 µg COMC/quilA
5	38	Challenge control
6	7	Negative control

225 ewes pre-screened
189 selected for study



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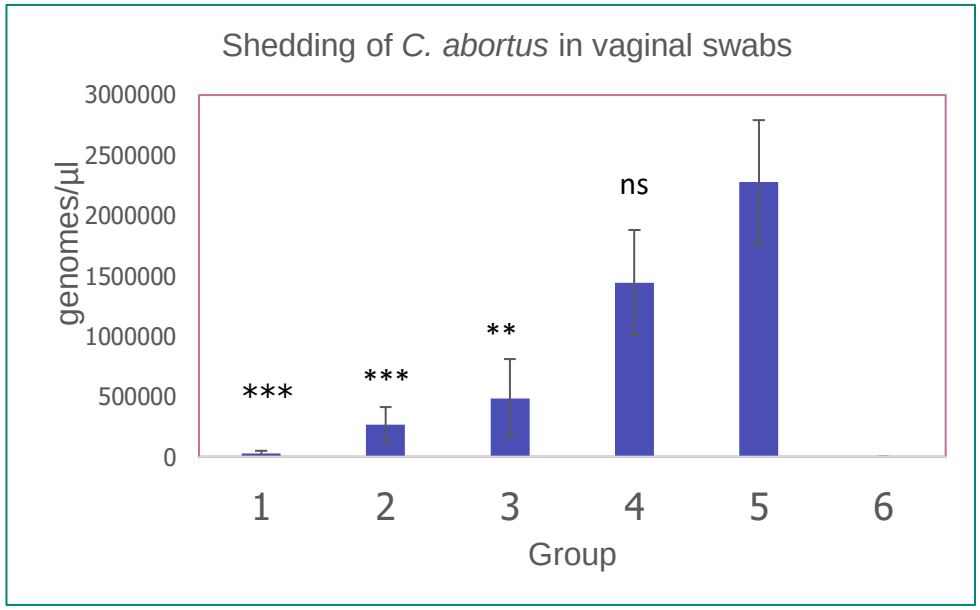
Vaccine trial 4

Group	Vaccine + infection status	Lambed	Aborted	% abortion	Pair-wise comparison to challenge control group 5
1	20µg Ag x ISA 70 VG	36	1	2.7%	p = 0.0228
2	2.5µg Ag x ISA 70 VG	31	1	3.1%	p = 0.0228
3	2.5µg Ag x ISA 61 VG	34	3	8.1%	p = 0.0228
4	2.5 µg Ag x QuilA	29	9	23.7%	p = 0.3067
5	Challenge control	24	14	36.8%	n/a
6	Negative control	7	0	0%	n/a



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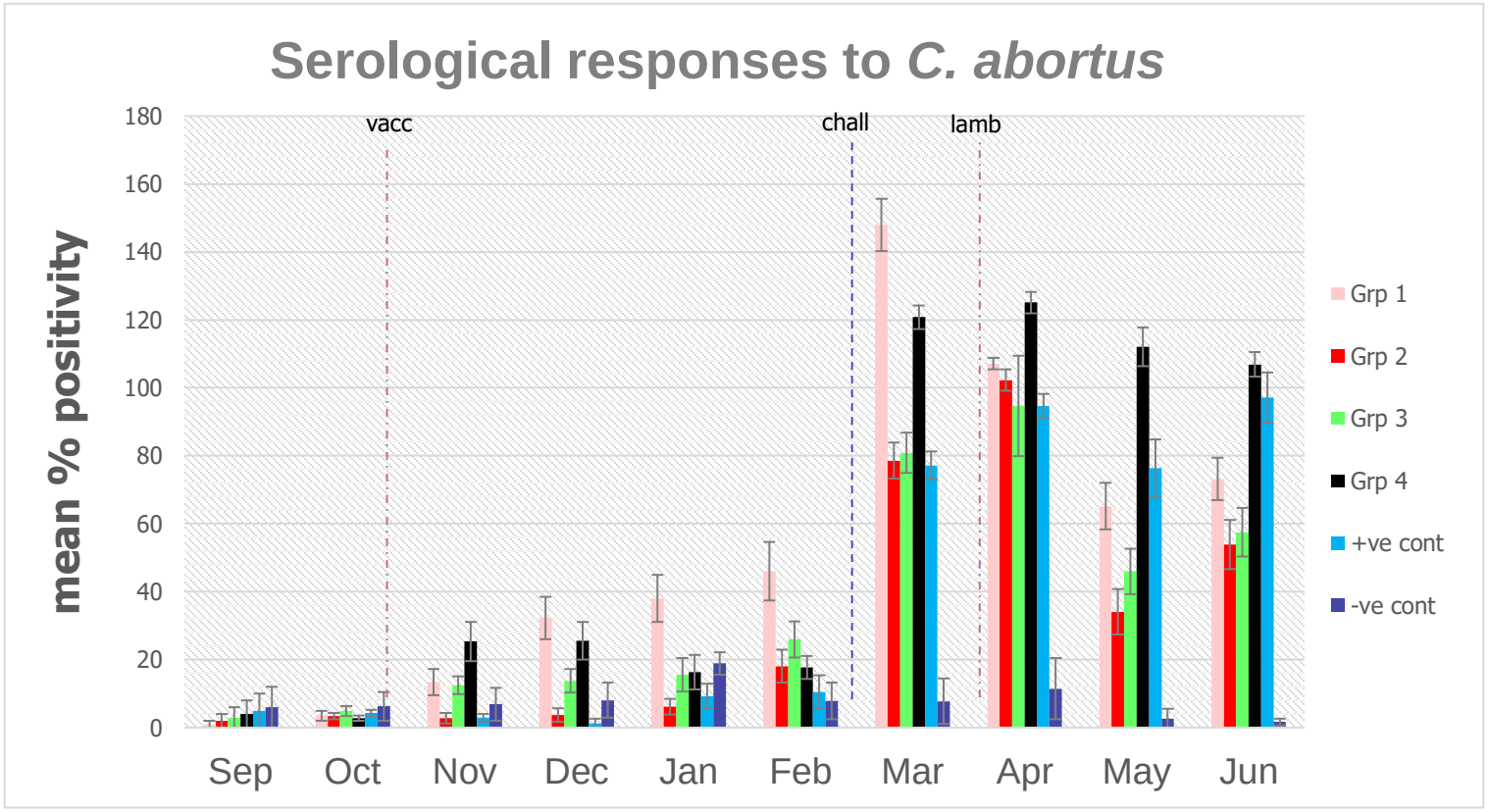
Vaccine trial 4: serological responses



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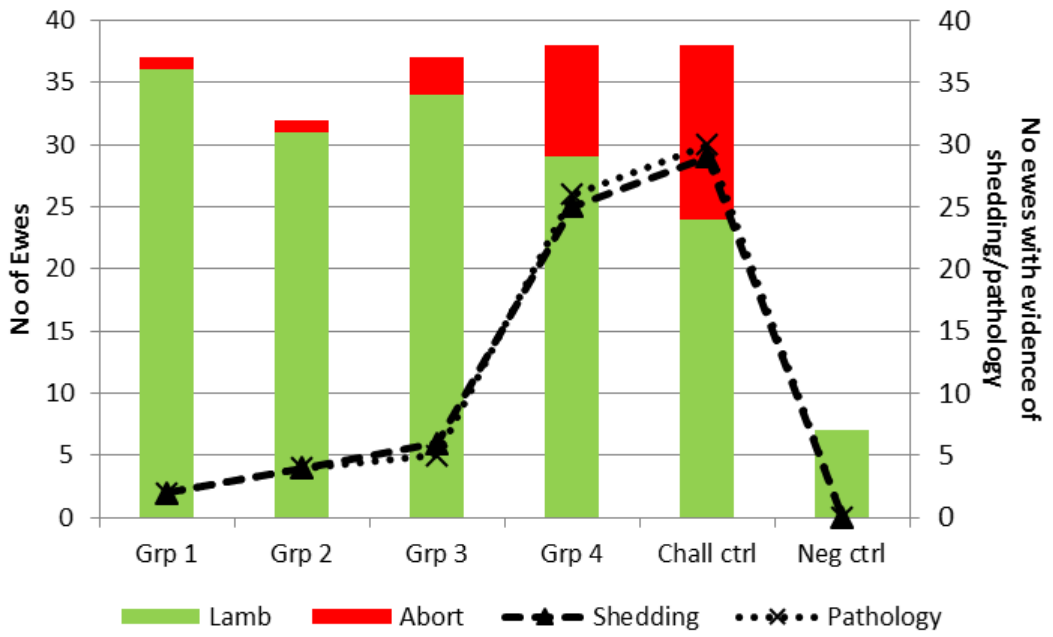
Vaccine trial 4

- Single dose of COMC Ag
- Adjuvant comparison
- Grp 1 (20µg/ISA70VG), Grp 2 (2.5µg/ISA70VG), Grp 3 (2.5µg/ISA61VG), Grp 4 (2.5µg/QuilA), Grp 5 (chall ctrl), Grp 6 (Neg ctrl)
- *Grp 1 and Grp 2 lowest abortions/least shedding*
- *QuilA did not protect*
- *ISA70VG adjuvant*



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Summary

- Vaccine development
  - Single shot vaccine
  - Dose
  - Adjuvant
  - Safe
  - Commercial viability
  - **Antigen production**
  - **Yield/cost**
  - **Commercialisation**

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Thanks for listening!
Questions?