Histopathological analysis of placental lesions caused by Chlamydia abortus 1B vaccine strain in vaccinated ewes.

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

[1] Moredun Research Institute, Pentlands Science Park, Edinburgh, UK; [2] Royal (Dick) School of Veterinary Studies, University of Edinburgh, UK; [3] Biomathematics & Statistics Scotland, Edinburgh, UK, [4] Instituto Nacional de Tecnología Agropecuaria, Argentina .

Background

Chlamydia abortus is one of the most diagnosed causes of infectious abortion in small ruminants. Infections can be controlled using the live, attenuated *C. abortus* strain 1B vaccine, which has been associated with infection and abortion in animals. This study aimed to compare the severity and the distribution of lesions caused by this vaccine strain (vt) with those resulting from a wild-type (wt) infection.

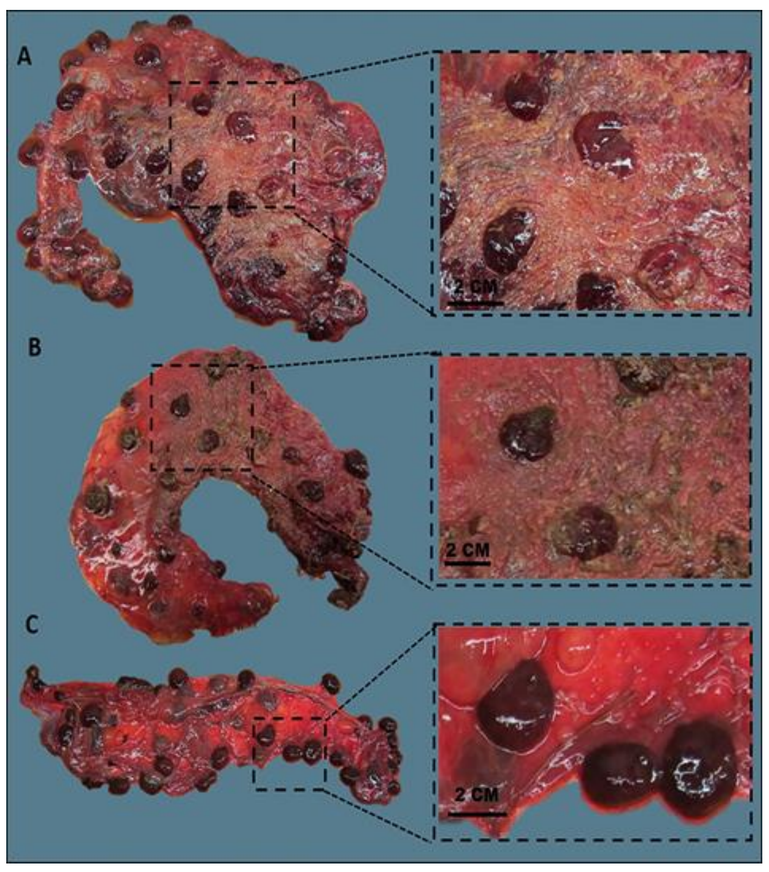

Figure 1. Placentas infected with vaccine-type (A) and wild-type (B) *C. abortus* strains, and negative control (C).

Material & Methods

Two grossly affected and 1B-positive (by qPCR and RFLP analysis) placentas from a vaccinated sheep flock were analysed. Histopathological lesions and immunohistochemical labelling (IHC) were graded by layer, cotyledonary trophoblast (CT) and intercotyledonary trophoblast (ICT) layers, and cotyledonary mesenchyme (CM) and intercotyledonary mesenchyme (ICM), scored from 0 to 5, according to their severity and proximity to the main blood vessels (Proximal: Px; Distal: D). Pathology in the vt infected placentas was compared with that in two wt infected placentas. Datasets generated for observed histological and pathological features were sorted according in parameters and analysed using principal component analysis (PCA).

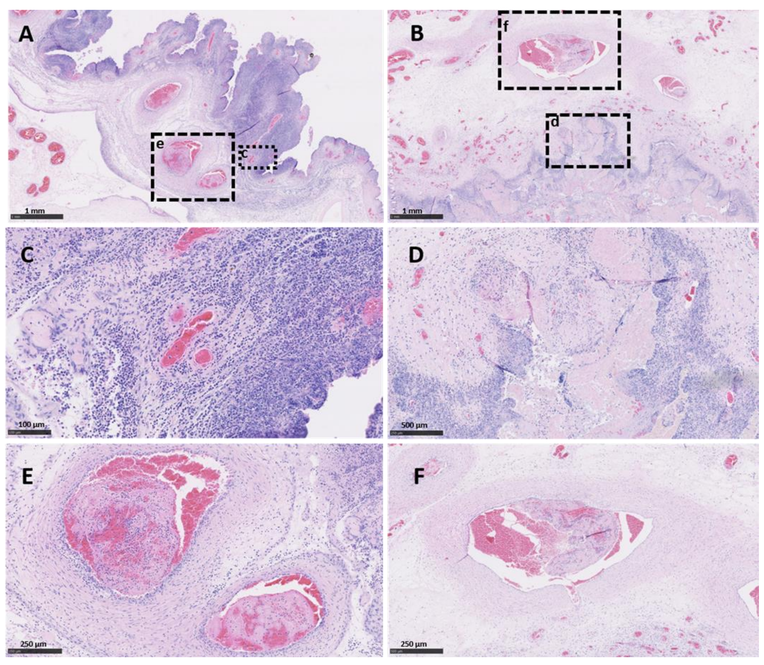

Figure 2. . Histopathological changes in the placentas of sheep infected with *C. abortus* vaccine-type (A, C and E) and wild-type strains (B, D and F) . Note the necrosuppurative placentitis with suppurative infiltration and fibrinoid necrosis of the trophoblast cells (expanded in C and D), and partially occlusive thrombosis of the artery (expanded in E and F).

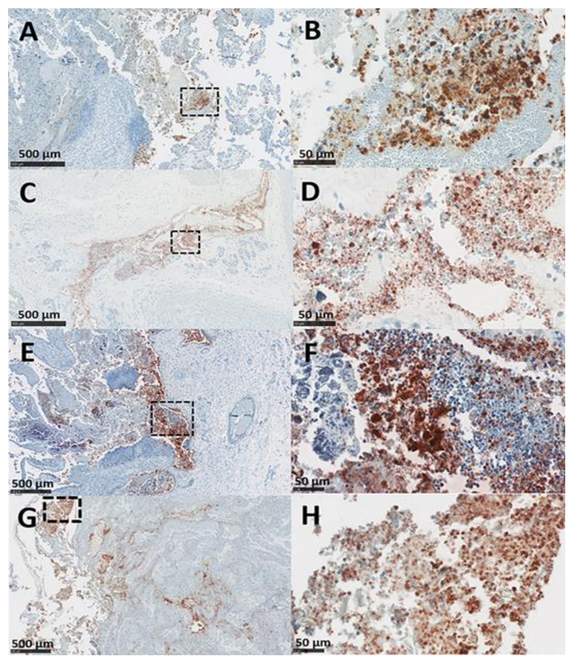

Figure 4. Immunohistochemical detection of chlamydial antigen in ovine placentas. Both vt- (A-D) and wt-placentas (E-H) were labelled by IHC using genus-specific anti-LPS mAb 13/4 and counterstained with haematoxylin.

Area	Location	Group	Epithelium and mesenchyme												Blood vessels																					
			N			PMNI			MI			TMIHC			VEA			VN			VMI			VPMNI			VT			VIHC							
			Q1	Med	Q3	Q1	Med	Q3	Q1	Med	Q3	Q1	Med	Q3	Q1	Med	Q3	Q1	Med	Q3	Q1	Med	Q3	Q1	Med	Q3	Q1	Med	Q3	Q1	Med	Q3					
CT	D	wt	2	3	3	2	3	3	2	2	2	1	2	3	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0					
		vt	3	3	4	3	3	4	2	3	3	3	3	4	0	0	0	0	0	0	0	0	2	0	0	2	0	0	2.8	0	0	0					
		neg	2	2	3	2	2	3	2	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0					
CT	Px	wt	2.8	3	4	2	3	4	2	2	3	1	2	3	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0					
		vt	4	4	5	3	4	5	3	3	4	3	3	4	0	0	0	0	2	4	0	0	2	0	2	3	0	0	0	0	0	0					
		neg	1	2	3	2	3	3	2	2	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0					
ICT	D	wt	1	2	2.8	1	2	2.8	1	2	2	0	1	3	0	0	0	0	0	0	0	0	0.8	0	0	0	0	0	0	0	0	0					
		vt	2	3	5	2	3	5	2	2	3	1.2	3	3.8	0	0	0	0	0	2.8	0	0	2	0	1	3	0	0	0	0	0	0					
		neg	0	0	2	0	0	2	2	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0					
ICT	Px	wt	2	2	3	1	2	2	1	2	2	0	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0					
		vt	3	5	5	2	5	5	2	3	4	2	3	4	0	0	0	0	4	5	0	0	2	0	2	5	0	0	0	0	0	0					
		neg	0	0	1	0	0	0	1	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0					
CM	D	wt	0	0	2	1	2	3	1	2	2	0	2	2.8	0	2	2	0	0	1.8	0	2	3	0	2	3	0	0	2	0	0	0					
		vt	0	0	1	2	2	3.8	2	2	3	0	2	3	0	0	2	0	0	0.8	2	2	3	1.2	2	3	0	1	3	0	0	0					
		neg	0	0	0	2	2	2	2	2	2	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0					
CM	Px	wt	0	0	0	2	2	3	2	2	2	0	0	2	0	0	2	0	0	0	0	2	2	0	1	2	0	0	2	0	0	0					
		vt	0	2	3	2	3	4	2	3	4	0	0	2	0	0	2	0	0	2	1	2	3	0	2	4	0	0	2	0	0	0					
		neg	0	0	0.5	1	2	2	2	2	2	0	0	0	0	0	0	0	0	0	0	2	2.5	0	0	2	0	0	0	0	0	0					
ICM	D	wt	0	1	3	2	2	3	2	2	3	0	0.5	2	0	0.5	2	0	0	1.8	1	2	2	0	2	2	0	0	0.8	0	0	0					
		vt	0	1	3	3	4	5	2	3	3	2	3	3	0	0	2	0	0	3	2	3	3	2	3	4	0	2	3	0	0	0					
		neg	0	0	0	1	1	2	1	2	2	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0					
ICM	Px	wt	0	1.5	3	2	2	3	2	2	2.8	0	1	2.8	0	0	1.8	0	0	0	2	2	3	0	2	3	0	0	1.8	0	0	0					
		vt	2	3	4	3	4	5	2	3	5	0	2	3	0	0	2	0	3	3	2	3	4	2	3	5	0	2	3	0	0	0					
		neg	0	0	0	1	2	2	1	2	2	0	0	0	0	0	0	0	0	0	0	0	2	0	0	2	0	0	0	0	0	0					

Figure 4. Summary statistics of the score data by area, location and treatment group for wild type (wt), vaccine type (vt) and negative control (neg) samples. Abbreviations Q1, Med and Q3 indicate first quartile, median and third quartiles, respectively.

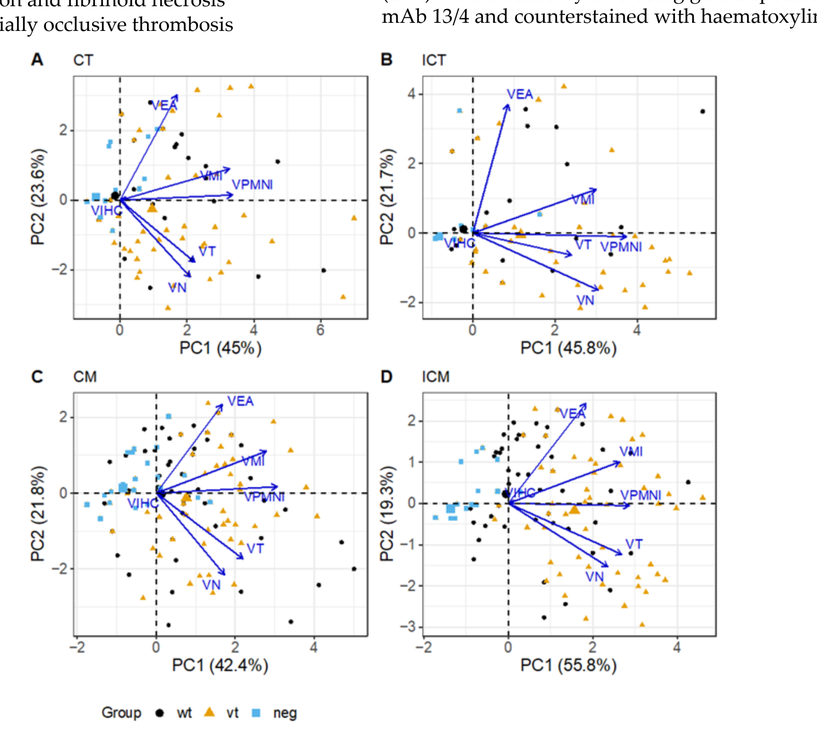

Figure 5. PCA biplots for trophoblast and mesenchyme layers in cotyledonary (CT, CM) and intercotyledonary (ITC, ICM) areas. The parameters analysed are necrosis (N), infiltration of polymorphonuclear leukocytes (PMNI), infiltration of mononuclear cells (MI), and labelling of chlamydial LPS by IHC (TMIHC).

Results

Histopathologically, the lesions in both vt and wt-placentas presented as typical multifocal necrosuppurative placentitis, associated with vasculitis, mural necrosis, and thrombosis (Fig 2). IHC for *C. abortus* revealed an intense labelling with a multifocal distribution in most cotyledons in both vt and wt placentas(Fig 3). Summary of the statistic of the score data for each pathological parameter by area (CT, ICT, CM, ICM), location (Px, D), and treatment group indicated by a change in colour gradation from green through to red (scores 0 to 4 or 5), there is a clear distinction between what is observed in the chorionic epithelium (trophoblast) and mesenchyme compared to the blood vessels, with the first showing higher scores overall (Fig 4).

Comparison of the pathological lesions between vt and wt by PCA revealed a similar distribution and severity, revealing a strong association with features such as necrosis and inflammatory infiltration between vt and wt placentas (Fig 5) . A weaker association with IHC was observed.

Conclusions

This study analysed the distribution and severity of pathology resulting from infection of the ovine placenta with the *C. abortus* live 1B vaccine strain in comparison to that resulting from a typical wt strain.

This was achieved using a novel grading system of scoring for pathological parameters and objectively analysed by PCA. The analysis provides detailed evidence that the commercial live vaccine strains can cause EAE lesions indistinguishable from those observed in the field strains, expanding our knowledge on the pathogenesis and role of the vaccine strain in causing disease. Furthermore, the approach taken may have applicability to other reproductive pathogens and future studies on placental pathology and pregnancy outcome.

THE UNIVERSITY of EDINBURGH
The Royal (Dick) School of Veterinary Studies

INTA

Ministerio de Agricultura, Ganadería y Pesca
Argentina

Moredun Research Institute