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

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ABSTRACT

Chlamydophila abortus, the agent of ovine enzootic abortion (OEA), is a major cause of lamb mortality worldwide. Disease can be controlled through the use of vaccines based on the 1B temperature-sensitive mutant strain of *C. abortus*. This study investigated suspected OEA cases across Scotland for the presence of the 1B strain by analysis of recently identified unique point mutations (9). Thirty-five cases were *C. abortus*-positive and 14 came from vaccinated flocks. Analysis of single nucleotide polymorphisms by PCR-RFLP and sequence analysis revealed the presence of point mutations consistent with the presence of the 1B vaccine strain in 5 of these 14 samples. Quantitative real-time PCR revealed comparable numbers of genome copies of the 1B strain in infected placentas to those present following wild-type infection. This study is the first to demonstrate the presence of the 1B vaccine strain in the placentas of OEA cases and suggests a probable causal role in the disease.

1. Introduction

Chlamydophila abortus is the aetiological agent of ovine enzootic abortion (OEA), the single most common infectious cause of ovine abortion in the UK and a major cause of lamb mortality throughout Europe. In addition, *C. abortus* is zoonotic and poses a considerable risk to the health of pregnant women [1]. Ewes infected with *C. abortus* prior to pregnancy exhibit no overt clinical signs of infection. Although ewes may exhibit a vaginal discharge 1–2 days prior to abortion occurring, usually the first clinical manifestation of disease is abortion or the birth of stillborn or weak lambs in the last 2–3 weeks of gestation [2]. These discharges along with those occurring following abortion or lambing, as well as the infected placentas, fetuses, and coats of lambs are the major sources of infection for other susceptible animals [1].

Disease control strategies can include the use of antibiotic therapy, although this is less effective when infection and pathology are more established [2]. Similarly the latent nature of infection makes serological detection prior to abortion very difficult [3]. More effective control can be accomplished through vaccination [4]. Currently the only commercial OEA vaccines licensed and available in the UK are based upon the 1B mutant strain of *C. abortus* (Enzovax®, Intervet/Schering-Plough Animal Health, UK; CEVAC Chlamydia®, CEVA Animal Health Ltd, UK). The 1B strain was originally derived from the virulent field strain AB7 by nitrosoguanidine mutagenesis as a temperature-sensitive (TS) mutant *in vitro*, due to a reduced growth rate at 39.5 °C (body temperature of sheep) and increased thermolability at 51 °C compared to its parent strain AB7 in plaque assays [5].

Although the 1B vaccine strain reduces the incidence of abortion and shedding in sheep and goats [6–8] it does not confer complete protection and some vaccinated animals do still abort. The differential detection of the 1B strain in vaccinated animals that have aborted, relying on a low multiplication rate at 39 °C and thermolability at 51 °C [5], is both difficult and time-consuming. Recently, comparative genomic analysis of the 1B and parental AB7 strains identified 22 single nucleotide polymorphisms (SNPs) unique to the mutant strain [9] and based on these results three vaccine-specific PCR-RFLP markers were recently developed [10,11]. The purpose of this study was to evaluate specific molecular tests based on some of these SNPs and markers, as well as their use in discriminating between vaccine and wild-type field strains of *C. abortus* in cases of OEA.

2. Materials and methods

2.1. Sample collection, flock histories and isolation of genomic DNA

Placentas from suspected cases of OEA were submitted to the Scottish Agricultural College Consulting, Veterinary Services (SACCVS) Disease Surveillance Centres (DSCs) (Aberdeen,

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Table 1
Clinical and OEA history of field cases.

Region	Farm	No. of affected[a]	Field Case	Vaccine	Date of vaccination	Origin of replacements	OEA history
A	a	12	#1	Enzovax	Autumn 2008	Homebred	No previous history of abortion
	b	12	#2	CEVAC Chlamydia	Autumn 2008	OEA accredited flock	NA
B	c		#3	Enzovax	August 2008	NA	OEA diagnosed in spring 2008
C	d	NA	#4	Enzovax	Autumn 2008	Bought in; non-accredited source	OEA diagnosed in spring 2008
	e	11	#5	Enzovax	Since 2006	Homebred	Sporadic abortions since 2005
	f	30	#6	Enzovax	Autumn 2008	Non-accredited source and homebred	No previous history of abortion
D	g	Large no.	#7	Enzovax	Autumn 2008	Bought in	Abortions in 2008 and before
E	h	5	#8	Enzovax	Autumn 2008	Bought in	NA
	i	6	#9	Enzovax	Autumn 2008	Homebred	NA
F	j	3	#10	Enzovax	Since 2001	Bought in; neighbour	OEA first diagnosed spring 2001
	k	6	#11	Enzovax	Autumn 2008	Bought in; non-accredited sources	OEA first diagnosed spring 2008
	l	6	#12	Enzovax	Since 2004	Homebred	OEA first diagnosed 2004
	m	2	#13	CEVAC Chlamydia	Autumn 2008	Bought in	New flock established May 2008; source farm known to have OEA in 2000, replacements homebred and vaccinated with Enzovax since 2000
	n	3	#14	Enzovax	Since autumn 2006	Homebred and bought in	OEA diagnosed prior to 2005; Mydiavac until autumn 2005
F	NA	NA	#15–17	Enzovax	Autumn 1993	NA	Unknown; aborted in spring 1994

NA, information not available.
[a] Information submitted by farmers, total number up to the time of submission.

Ayr, Dumfries, Edinburgh, Perth, St. Boswells, Inverness and Thurso) in spring 2009. Placentas were macroscopically examined for gross pathology typical of OEA [1,2] and smears prepared for detection of chlamydiae by modified Ziehl–Neelson (mZN) staining [12]. Representative cotyledons from these tissues were frozen with and without chlamydia transport medium (sucrose–phosphate–glutamate medium supplemented with fetal bovine serum, antibiotics and fungal inhibitor [13]) at −70 °C prior to transportation to Moredun Research Institute for analysis. Tissue samples were finely chopped using sterile blades and genomic DNA extracted using a DNeasy® Blood & Tissue Kit (Qiagen, Crawley, West Sussex, UK). All samples were eluted in 200 μl of supplied TE buffer (10 mM Tris–HCl, 0.5 mM EDTA, pH 9.0) for analysis. Routinely, fetal fluid samples were also tested for *Toxoplasma gondii*, and bacteriological analysis carried out on fetal stomach contents for *Campylobacter*, *Salmonella* and *Listeria* species. Where possible, the number of animals affected on each farm, full OEA flock histories, including vaccination history, vaccine type and source of replacement ewes, were obtained for each case investigated (Table 1). Manufacturer's instructions for administration of the live vaccine were adhered to in all cases.

In addition, archived DNA samples originating from sheep placental samples that had been submitted to Moredun for testing in 1994 from the St. Boswells DSC were also included in the study. Six ewes, all of which had been vaccinated with Enzovax®, had aborted their lambs in spring 1994 and *C. abortus* was isolated from all of them. At that time the growth of the isolates was compared at 37 °C and 40 °C, as well as thermolability at 51 °C, in order to determine whether they were wild-type strains or originated from the vaccine. Genomic DNA samples from three of these six isolates were found in our archived DNA collection, where they had been stored at −20 °C since the testing, the results of which according to our records had been ambiguous.

2.2. Real-time polymerase chain reaction

Genomic DNA samples were analysed by *C. abortus*-specific real-time PCR using primers and probes based on the *ompA* gene, as described previously [14]. Briefly, the PCR was performed in a total volume of 25 μl, containing 1 μl eluted DNA (approximately 100 ng), 12.5 μl of 2× TaqMan® universal master mix (Applied Biosystems, Warrington, UK), 300 nM final concentration of each primer and 250 nM final concentration of fluorescent probe. Amplification was performed on an ABI Prism 7000 sequence detection system (Applied Biosystems, UK), following manufacturer's standard protocols. Thermal cycling conditions were 50 °C for 2 min and 95 °C for 10 min, followed by 45 cycles of 95 °C for 15 s and 60 °C for 1 min.

Quantification of the number of genome copies present in each sample per microgram of total tissue DNA was calculated as previously described [14], using *C. abortus* S26/3 genomic DNA to construct a standard curve. Spiked and negative control samples were included to control for DNA extraction efficiency, as well as potential contamination and inhibitory factors. The number of genomes present in placental samples was compared across vaccine groups. Prior to statistical analysis genome numbers were