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Propagation of the Israeli vaccine strain of *Anaplasma centrale* in tick cell lines

Lesley Bell-Sakyi[a,*], Ana M. Palomar[a,b], Emma L. Bradford[a,c], Varda Shkap[d]

[a] The Pirbright Institute, Ash Road, Pirbright, Woking, Surrey GU24 0NF, UK
[b] CIBIR, C/ Piqueras, 98, Logroño 26006, Spain
[c] Institute of Biological and Environmental Sciences, University of Aberdeen, Tillydrone Avenue, Aberdeen AB24 2TZ, UK
[d] Kimron Veterinary Institute, Bet Dagan, 50250, Israel

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ABSTRACT

Anaplasma centrale has been used in cattle as a live blood vaccine against the more pathogenic *Anaplasma marginale* for over 100 years. While *A. marginale* can be propagated *in vitro* in tick cell lines, facilitating studies on antigen production, immunisation and vector-pathogen interaction, to date there has been no *in vitro* culture system for *A. centrale*. In the present study, 25 cell lines derived from 13 ixodid tick species were inoculated with the Israeli vaccine strain of *A. centrale* and monitored for at least 12 weeks by microscopic examination of Giemsa-stained cytocentrifuge smears. Infection of 19 tick cell lines was subsequently attempted by transfer of cell-free supernate from vaccine-inoculated tick cells. In two separate experiments, rickettsial inclusions were detected in cultures of the *Rhipicephalus appendiculatus* cell line RAE25 28–32 days following inoculation with the vaccine. Presence of *A. centrale* in the RAE25 cells was confirmed by PCR assays targeting the 16S rRNA, *groEL* and *msp4* genes; sequenced PCR products were 100% identical to published sequences of the respective genes in the Israeli vaccine strain of *A. centrale*. *A. centrale* was taken through three subcultures in RAE25 cells over a 30 week period. In a single experiment, the *Dermacentor variabilis* cell line DVE1 was also detectably infected with *A. centrale* 11 weeks after inoculation with the vaccine. Availability of an *in vitro* culture system for *A. centrale* in tick cells opens up the possibility of generating a safer and more ethical vaccine for bovine anaplasmosis.

1. Introduction

Anaplasma centrale, first isolated from a heifer in South Africa in 1909 (Theiler, 1911; cited by Herndon et al., 2013), has been used as a live blood vaccine to protect against bovine anaplasmosis caused by *Anaplasma marginale* for over 100 years. Currently the *A. centrale* vaccine is used to protect cattle in several African, South American and Middle Eastern countries including Israel. Production of the vaccine involves infecting splenectomised cattle with *A. centrale* stabilate and harvesting large volumes of blood from them when the rickettsaemia reaches a suitable level (OIE, 2014). Live blood vaccines have a number of disadvantages including risk of co-transmission of other ruminant pathogens, risk of haemolytic disease in calves born to vaccinated dams and requirement for a stringent cold chain. While an *in vitro* culture system for *A. marginale* in cell lines derived from the tick *Ixodes scapularis* has been available for nearly two decades (Munderloh et al., 1996), and has resulted in exponential progress in knowledge and understanding of this pathogen, to date it has not been possible to propagate *A. centrale in vitro*. Ability to cultivate *A. centrale in vitro* would open up the possibility of producing vaccine antigen without the need to splenectomise, infect and exsanguinate cattle.

The present study was carried out with the aim of establishing *in vitro* culture of the Israeli vaccine strain of *A. centrale* in one or more tick cell lines, taking advantage of the availability in the Tick Cell Biobank (http://www.pirbright.ac.uk/research/Tickcell/Default.aspx) of multiple cell lines derived from five ixodid tick genera.

2. Materials and methods

2.1. Tick cell lines

A panel of 32 tick cell lines derived from 14 ixodid tick species (Table 1) were tested for ability to support infection and replication of *A. centrale*. The cell lines were grown at either 28 °C or 32 °C in

* Corresponding author.
E-mail addresses: lesley.sakyi@pirbright.ac.uk (L. Bell-Sakyi), ampalomar@riojasalud.es (A.M. Palomar), dr01elb14@abdn.ac.uk (E.L. Bradford), shkapv@int.gov.il (V. Shkap).

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Table 1
Tick cell lines tested for ability to support growth of *Anaplasma centrale*. The original references for the tick cell lines are cited by Alberdi et al. (2012) except where indicated. *A. centrale* was inoculated (X) as either diluted vaccine or as clarified supernate from already-infected tick cells.

Tick species	Cell line	Culture medium/incubation temperature	Inoculum	
			From vaccine	From tick cells
Amblomma americanum	AAE2	L-15B300/32 °C		X
	AAE12	L-15B300/32 °C		X
Amblyomma variegatum	AVL/CTVM13	L-15/L-15B/32 °C		X
	AVL/CTVM17	L-15/H-Lac/L-15B/32 °C	X	
Dermacentor albipictus	DALBE3	L-15B300/32 °C	X	
Dermacentor andersoni	DAE15	L-15B300/32 °C	X	X
	DAE100T	L-15B300/32 °C	X	X
Dermacentor nitens	ANE58	L-15B300/32 °C	X	
Dermacentor variabilis	DVE1	L-15B300/32 °C	X	X
Hyalomma anatolicum	HAE/CTVM8	L-15/H-Lac/32 °C		X
	HAE/CTVM9	L-15/MEM/32 °C	X	
Ixodes ricinus	IRE/CTVM19	L-15/28 °C	X	
Ixodes scapularis	IDE2	L-15B300/32 °C	X	X
	IDE8	L-15B/32 °C	X	X
	ISE6	L-15B300/32 °C	X	X
	ISE18	L-15B300/32 °C	X	
Rhipicephalus appendiculatus	RAE/CTVM1	L-15/28 °C	X	X
	RAN/CTVM3	H-Lac/28 °C		X
	RAE25[a]	L-15B/32 °C	X	X
	RA243	L-15/32 °C	X	X
Rhipicephalus evertsi	REE/CTVM29	L-15/28 °C		X
	REE/CTVM31	L-15/MEM/28 °C	X	
	REN/CTVM32[b]	L-15/H-Lac/28 °C	X	
Rhipicephalus sanguineus	RSE8	L-15/L-15B/32 °C		X
	RML-RSE[c]	L-15/MEM/28 °C	X	
Rhipicephalus (*Boophilus*) *decoloratus*	BDE/CTVM16	L-15/28 °C	X	X
Rhipicephalus (*Boophilus*) *microplus*	BME/CTVM2	L-15/28 °C	X	X
	BME/CTVM5	L-15/MEM/28 °C	X	
	BME/CTVM6	L-15/28 °C	X	
	BME/CTVM23	L-15/32 °C	X	X
	BME/CTVM30	L-15/MEM/28 °C	X	
	BmVIII-SCC	L-15/MEM/32 °C	X	

[a] Kurtti and Munderloh (1982).
[b] Bell-Sakyi (unpublished); derived from developing adult *R. evertsi* ticks kindly supplied in 2010 by Dr. Ard Nijhof, then of Utrecht Centre for Tick-borne Diseases, Utrecht University, The Netherlands.
[c] Previously deposited in the Tick Cell Biobank as *D. variabilis* embryo-derived cell line RML-15 (Yunker et al., 1981). However sequencing of a fragment of the 16S rRNA gene (Black and Piesman, 1994) revealed that the cell line was actually derived from *R. sanguineus* (data not shown). Three embryo-derived *R. sanguineus* cell lines were established in the same laboratory as RML-15: RML-21, 22 and 23 (Yunker et al., 1984, 1987). As it is now impossible to determine which of the three cell lines was used in the present study, it is here designated RML-RSE.

sealed flat-sided culture tubes (Nunc) containing 2.2 ml of complete culture medium (L-15, H-Lac, L-15B, L-15B300, L-15/MEM, L-15/H-Lac, L-15/L-15B or L-15/H-lac/L-15B as described previously (Munderloh and Kurtti, 1989; Munderloh et al., 1999; Bell-Sakyi, 2004). Prior to infection with *A. centrale* the supernatent medium was removed from each tube, the cell monolayer was washed once with 1 ml of L-15B medium supplemented with 10% FCS, 10% TPB, 0.1% bovine lipoprotein (MP Biomedicals), 2 mM L-glutamine, 15 mM HEPES and 0.1% $NaHCO_3$ (ACGM) to remove traces of antibiotics and 2 ml of ACGM was added to the tube. For cultures receiving blood vaccine, ACGM was further supplemented with 5 μg/ml Amphotericin B (ACGMA).

2.2. Inoculation of tick cell lines with Anaplasma centrale-infected bovine erythrocytes

The Israeli *A. centrale* blood vaccine comprising bovine erythrocytes with *A. centrale* rickettsaemia of 20%, cryopreserved with 5% DMSO as 1.8 ml aliquots containing 1×10^8 infected erythrocytes, was prepared at the Kimron Veterinary Institute and stored in the vapor phase of a liquid nitrogen refrigerator prior to and following transfer on dry ice to the Pirbright Institute. For inoculation onto tick cell lines, a vial of vaccine was thawed rapidly by immersion in a 37 °C water bath and the contents were immediately diluted in 9 ml of ACGMA at room temperature. Aliquots of 0.6–0.7 ml were immediately added to tubes of tick