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Growth Yields of Four *Coxiella burnetii* Isolates in Four Different Cell Culture Lines

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ABSTRACT

Although *Coxiella burnetii* is considered to be an obligate intracellular bacterium and grows in embryonated eggs, laboratory animals and cell culture, recently it has been grown in cell-free media and on agar plates. This current study was conducted to compare four cell lines for their yield of *C. burnetii.* Four different isolates of *C. burnetii* (Henzerling, Arandale, Cumberland and Timony) were grown in DH82, L929, Vero and XTC-2 cell lines. The DH82 and XTC-2 cells lines produced the highest *C. burnetii* yield which was slightly less than the yields achieved in recently published studies using cell free media. The Arandale isolate of *C. burnetii* produced a significantly higher yield in DH82 cells compared to XTC-2 cells ($P < 0.03$).

Keywords: Bacterial Yield; Cell Culture; *Coxiella burnetii*; Intracellular Bacteria

1. Introduction

Q Fever is a worldwide zoonosis caused by *Coxiella burnetii* and serological testing by immunofluorescence assay (IFA) is generally used for diagnosis. *C. burnetii* was traditionally cultured in embryonated eggs or laboratory animals such as guinea pigs and mice. The use of cell culture has permitted the growth of *C. burnetii*, in flasks or multi-welled trays containing a monolayer of eukaryotic host cells [1]. Traditionally, *C. burnetii* has been considered an obligate intracellular bacterium. However, *C. burnetii* was recently grown without host cells [2].

In cell culture the infection does not generally destroy the host cells and infected cells have the same cell cycle progression as uninfected cells. This is a result of asymmetric division of infected cells producing one infected and one uninfected daughter cell. This ability of *C. burnetii* has allowed it to persistently infect cell cultures for over two years without the addition of uninfected cells [3]. The infected cell monolayer exhibits cytopathic effect (CPE) at the same rate as uninfected cultures. Thus infection of the culture must be observed through the use of other methods such as IFA or polymerase chain reaction (PCR). The optimal growth of *C. burnetii* is important if large numbers of bacteria are required for protein or DNA studies or vaccine production. In this study four different cell lines were compared to determine which produced the greatest yield of *C. burnetii*. The cell lines chosen for this study included cell lines used to grow *C. burnetii* previously; Vero (African green monkey epithetlial cells) [4] and L929 (mouse fibroblast cells) [5,6] and two other cell lines currently used in our laboratory; DH82 (canine macrophage cells) and XTC-2 (South African clawed frog epithelial cells). This study compares the yields obtained in cell lines to the yields obtained in cell-free media [7].

2. Materials and Methods

Vero, DH82 and L929 cells were grown in 10ml RPMI (Gibco, Australia) supplemented with 10% new born calf serum (NBCS) (Gibco, Australia) and 1% L-glutamine (Gibco, Australia). Cell lines were incubated at 35˚C with 5% CO_2. The XTC-2 cell line was grown with 10 ml Leibovitz L-15 (Gibco, Australia) media supplemented with 10% NBCS (Gibco, Australia), 0.4% tryptose phosphate broth (Oxoid, England) and 1% L-glutamine (Gibco, Australia) and incubated at 28˚C. These were inoculated with suspensions of antigenic phase I *C. burnetii* of the Henzerling isolate (homogenised infected egg yolk sack, courtesy of Commonwealth Serum Laboratories CSL, Australia) and three Australian isolates Arandale, Cumberland and Timony (homogenised infected spleens from severe combined immunodeficiency [SCID] mice). The suspensions (0.5 mL) were first diluted in 9.5 mL of

Hanks' balanced salt solution (HBSS, Gibco, Australia) and filtered through a 0.45 μm filter to reduce the amount of host material. An aliquot of the filtrate (0.8 ml) was added to each flask. Two flasks of each confluent cell line were inoculated with each *C. burnetii* isolate. Cultures had fortnightly changes of media. At six weeks post infection it was assumed that maximal growth had occurred and the monolayer was harvested by scraping, pelleted and resuspended in 1 ml PBS. All cultures growing *C. burnetii* were performed in bio-containment level 3 laboratory at the Department of Microbiology, John Hunter Hospital, Newcastle.

DNA was extracted from a 200 μl aliquot of this suspension using the Qiagen QIAamp DNA Mini Kit (Qiagen, Germany) following the manufacturers instructions. Samples were then analysed by a real time PCR assay [8] targeting the *Com*1 gene, which codes for a highly conserved 27 kDa outer membrane protein. The cycling threshold (C_t) result was used to calculate the DNA concentration (μg/μl). This was achieved using a standard curved made with plasmids cloned with the resulting PCR amplicon as described previously [8].

3. Results

The results showed higher growth yields in DH82 cell line for two isolates (Henzerling and Arandale) and in XTC-2 cell line for the other two (Cumberland and Timony) (**Table 1**). The Arandale isolate grew to higher numbers in the DH82 cell line compared to the XTC-2 cell line (P = 0.03). Overall the XTC-2 cell line appeared to yield the highest concentration of *C. burnetii* DNA after six weeks of culture. To determine if the host cell density was proportional to the yield of intracellular bacteria the optical density (OD) of the cell monolayers harvested was determined (data not shown). While variation was found in OD between flasks this did not correlate with the yield of *C. burnetii* DNA. To compare these results with previous studies the data was converted into bacterial cell numbers per ml of media. The total yield of *C. burnetii* obtained was approximately 10^7/mL of media in a flask containing a monolayer of host cells and 10 mL of media.

4. Discussion

Obtaining *C. burnetii* isolates enables comparative studies to be made on different isolates. The yield of these bacteria produced in different cell lines is currently unknown. The findings of this study indicate that cell line DH82 and/or XTC-2 are best for growing *C. burnetii* to a high yield. The reasons for the preference of one cell line over another were not determined. This may be due to the ease with which *C. burnetii* can enter and multiply within that host cell, a feature of the *C. burnetii* strain itself, or the conditions under which it was grown; such as the lower temperature for the XTC-2 cell line or a component of the media.

The isolates used include the Henzerling isolate from an acute case of Q fever in Italy and which has been classified as group II [9]. The three other isolates used in this study were from acute cases of Q fever in Australia and were classified as group III (typing method from [10] (data not shown).

To determine if the lack of statistical significance between each cell line was due to high variability of host cell numbers, the optical density (OD) of the cells was determined (data not shown). It was presumed that a higher OD reading and a higher density of host cells would correlate with a higher yield as there would be more host cells with vacuoles full of bacteria. While variation was found in OD between flasks this did not correlate with the variability in the amount of *C. burnetii* DNA detected when compared across either isolate or cell line groupings.

Using these cell lines the total yield (in a flask containing host cells and 10 mL of media) was around 10^7/mL. This was less than when *C. burnetii* was grown without the use of host cells yeilding around 10^8/mL of media [7]. *C. burnetii* grown in cell lines consists of *C. burnetii* free in the media (having been released from their intracellular location) and in vacuoles within host cells in the monolayer. If the monolayer was growing in 1 mL of media (rather than 10 mL) the yield of bacteria per mL would have been the same as the cell free media. However, such a low media volume may not give optimal conditions for the host cells. Greater yields have been obtained through animal inoculation with severe combined immunodeficiency (SCID) mice producing 9×10^9/g of spleen (Michelle Lockhart, personal communication). However the use of animals is more costly to set up and maintain. The results of this study indicate that the cell free media may be preferable for producing *C. burnetii* with the added advantage of no contaminating host cells.

Table 1. Yield (μg/μl of DNA detected by Com1 PCR) of four isolates of *C. burnetii* grown in four different cell culture lines.

C. burnetii	Cell lines			
	DH82	L929	Vero	XTC-2
Henzerling	**5.5 (0.7)**	1.8 (0.1)	2.5 (1.1)	2.9 (0.1)
Arandale	**5.8 (0.1)***	4.5 (3.0)	3.0 (0.9)	3.7 (0.3)*
Cumberland	5.2 (3.2)	0.9 (0.6)	2.3 (0.2)	**6.6 (1.6)**
Timony	2.6 (0.6)	2.8 (0.4)	2.3 (1.0)	**9.4 (7.0)**
Average	4.8 (1.9)	2.5 (1.8)	2.5 (0.7)	**5.7 (3.9)**

One standard deviation is given in brackets; highest yield of DNA from each isolate is given in bold. Statistical differences (by student t-test) *P = 0.03.

Higher yeilds would be useful for some studies and in the case of vaccine production.

In the human host *C. burnetii* infects monocytes and macrophages. *Coxiella burnetii* enters the host cell by inducing endocytosis in phagocytes. Unlike other intracellular bacteria that either escape the phagosome into the cytoplasm or prevent the binding of the lysosomes to the phagosome, *C. burnetii* survives in the highly acidic environment within the phagolysosome [6]. The intracellular environment gives the bacteria access to the host cell's nutrients and molecular building blocks. *Coxiella* actively transports glucose, glutamate [11], proline [12] and other substrates from the intracellular environment. These transportation systems and the metabolism of nucleic acids and amino acids have been shown to be pH dependent [11-13]. The host-cell free media [2,7] appears to mimic this intra phagolysosome environment and reduces the need for growing and maintaining cell lines. We have shown that the use of cell lines produces a lower yield of *C. burnetii* than in cell free media [7].

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