Q fever – A continued threat to the military. Can antibiotic prophylaxis reduce the severity of disease?

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Background

Q fever is caused by the intracellular bacterium *Coxiella burnetii*. It is a zoonotic infection that is endemic worldwide, with bacteria concentrated in the birth products of ruminant animals. Infection is largely transmitted via the aerosol route. Either from infected birth products or from windborne spread of dessicated infected material. It was initially identified as a problem for the military during WWII with thousands affected during the campaign[1]. More recently Q fever has been recognised as a problem in UK troops returning from Afghanistan[2]. Clinical presentation is pleomorphic, with around 20% of patients exhibiting long term Q fever fatigue syndrome[3] and 1% developing chronic infection, usually endocarditis[4]. The risk of endocarditis is increased with valvular abnormality. In addition, the low infectious dose and environmental stability of *C. burnetii* has led to the classification as a CDC category B agent. There is no licenced vaccine in the UK against Q fever and antibiotic therapy for chronic infection can be lengthy and complex. Therefore, antibiotic prophylaxis to prevent these debilitating sequelae should be evaluated.

Doxycycline is the first line treatment for Q fever with quinolones being an alternative[5]. In this study, a range of antibiotics (doxycycline, ciprofloxacin, levofloxacin, co-trimoxazole) were tested *in vitro* and *in vivo*. In order to inform an efficacy study, *in vitro* assays were undertaken to determine the minimum inhibitory concentration for each antibiotic. (Figure 1a.1b.)

Finally, 100 A/J mice were challenged with *C. burnetii* via aerosol route and treated with 7 days of each antibiotic, starting either 24 h pre or 24 h post exposure. Mice were monitored for 14 days post-exposure, with body weight and clinical signs measured daily, organ weight at necropsy, and bacterial load within the spleen and lungs measured at the end of the study. Doxycycline and levofloxacin administered pre and post exposure significantly protected against body weight loss compared with the controls ($p<0.05$), whereas ciprofloxacin and co-trimoxazole provided no significant protection.

A.

Antibiotic	MIC (µg/ml) (broth dilution)	E-tests(µg/ml)
Doxycycline	0.004 - 0.16	0.16 – 0.23
Ciprofloxacin	1- 2	0.064 - 1.5
Levofloxacin	0.5 - 4	0.064 - 4
Moxifloxacin	1 - 4	0.19 - 1
Co-trimoxazole	16 - 32	32

B.

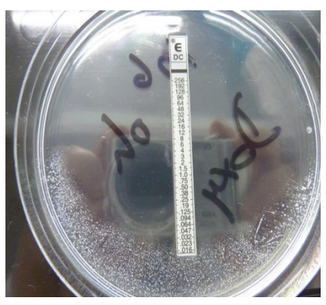


Figure 1a.Table summarising the MIC values for *C.burnetii* using the broth dilution and E-test methodology. All experiments were completed in triplicate. Figure **1b**. An example of the E-test results for *C.burnetii* on ACCM-2 agar. The results were read at 7 days by two independent observers.

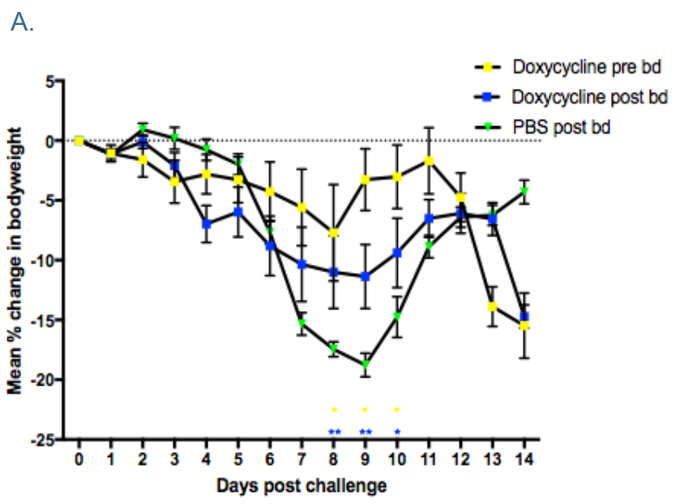
Pharmacokinetic and pharmacodynamic parameters were analysed in A/J mice, enabling selection of an appropriate antibiotic dose to closely mimic the human regimen (Figure 2).

A.

Drug	Mouse regimen	Route of administration	PK parameter required in humans	Human equivalent dose
Ciprofloxacin	22 mg/kg BD	IP	AUC = 23.3 mg/L/h	500mg BD
Levofloxacin	40 mg/kg OD	IP	AUC = 45.6 mg/L/h	500mg OD
Doxycycline	105 mg/kg BD	oral	AUC = 40 – 123 mg/L/h	100mg BD
Co-trimoxazole	48 mg/kg BD	oral	T > MIC for 2/3rd of time MIC = 2 µg/ml	960mg OD

Figure 2.Table summarising the antibiotic doses and routes of administration for the A/J mouse experiment using preceeding pharmacokinetic information in the A/J mouse in order to mimic the human dose received from standard antibiotic preparations. (IP – intraperitoneal, AUC – area under the curve, MIC – minimum inhibitory concentration).

A.



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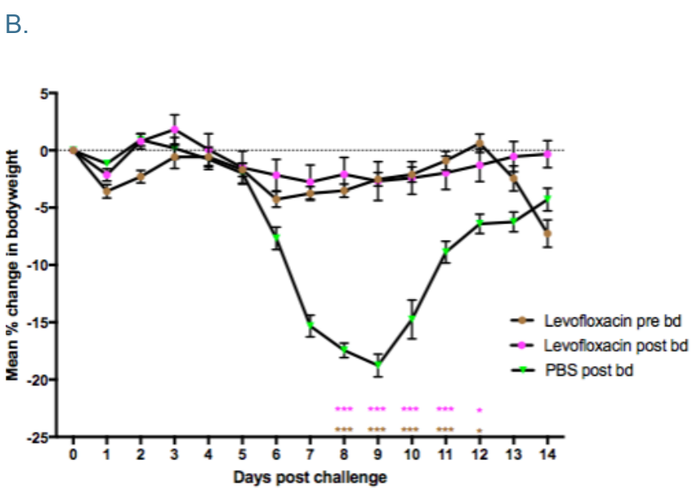

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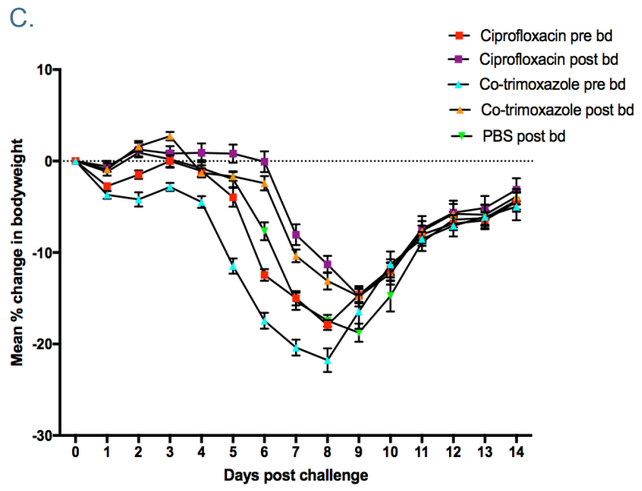

Figure 3. A/J mice weight loss curve as a percentage of initial body weight for **a**. doxycycline versus the control (phosphate buffered solution, PBS). **b**. Levofloxacin vs control **c**. co-trimoxazole & ciprofloxacin vs control. Doxycycline and levofloxacin provided significant protection against weight loss ($p<0.05$), wheareas ciprofloxacin and co-trimoxazole did not confer any weight loss benefit over the control group. (* $p<0.05$, ** $p<0.01$, *** $p<0.001$). All statistical analysis was performed using Graphpad Prism v7. A two-way random matching ANOVA was performed on the raw mouse weight data and compared for significance using Tukey's multiple comparisons tests.

Conclusions

- Doxycycline and levofloxacin pre and post exposure significantly protect against weight loss.
- Doxycycline prophylaxis delays weight loss until the antibiotics are completed.
- It is not known why co-trimoxazole and ciprofloxacin do not protect against weight loss. Work is ongoing to determine intracellular antibiotic concentration which might help us to understand why they were less effective.
- Future work will focus on extending the length of therapy to 14 days with post exposure therapy starting at day 5 post exposure, to replicate human Q fever where antibiotics are commenced when symptomatic.
- Doxycycline hyclate used in this experiment affected the A/J mouse gut leading to weight loss. Further studies will use doxycycline monohydrate instead.

References

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