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Comparative proteomic analysis of lung tissue from guinea pigs with leptospiral pulmonary haemorrhage syndrome (LPHS) reveals a decrease in abundance of host proteins involved in cytoskeletal and cellular organization

Simone Schuller[a,d,*], *Kjell Sergeant*[b], *Jenny Renaut*[b], *John J. Callanan*[a,c,e], *Caitriona Scaife*[c], *Jarlath E. Nally*[a,c,f]

[a]*University College Dublin, School of Veterinary Medicine, Belfield, Dublin 4, Ireland*
[b]*Luxembourg Institute of Science and Technology, Environmental Research and Innovation" (ERIN) department, 41, rue du Brill, 4422 Belvaux, Luxembourg*
[c]*Conway Institute for Biomolecular & Biomedical Research, Belfield, Dublin 4, Ireland*
[d]*Vetsuisse Faculty University of Bern, Länggassstrasse 128, 3012 Bern, Switzerland*
[e]*Ross University School of Veterinary Medicine, St Kitts and Nevis, West Indies*
[f]*Bacterial Diseases of Livestock Research Unit, National Animal Disease Center, Agricultural Research Service, Department of Agriculture, Ames, IA 50010, USA*

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ABSTRACT

Leptospiral pulmonary haemorrhage syndrome (LPHS) is a particularly severe form of leptospirosis. LPHS is increasingly recognized in both humans and animals and is characterized by rapidly progressive intra-alveolar haemorrhage leading to high mortality. The pathogenic mechanisms of LPHS are poorly understood which hampers the application of effective treatment regimes. In this study a 2-D guinea pig proteome lung map was created and used to investigate the pathogenic mechanisms of LPHS. Comparison of lung proteomes from infected and non-infected guinea pigs via differential in-gel electrophoresis revealed highly significant differences in abundance of proteins contained in 130 spots. Acute phase proteins were the largest functional group amongst proteins with increased abundance in LPHS lung tissue, and likely reflect a local and/or systemic host response to infection. The observed decrease in abundance of proteins involved in cytoskeletal and cellular organization in LPHS lung tissue further suggests that infection with pathogenic *Leptospira* induces changes in the abundance of host proteins involved in cellular

Abbreviations: ACN, Acetonitrile; APP, Acute phase protein; APS, Ammonium persulfate; ARDS, Adult/acute respiratory distress syndrome; ASB-14, Amidosufobetaine-14; d.p.i, Days post infection; DFM, Dimethylformamide; EMJH, Ellinghausen-McCullough-Johnson-Harris; H&E, Haematoxylin and eosin; H_2O_2, Hydrogen peroxide; HCl, Hydrochloric acid; HRP, Horseradish peroxidase; IFN-γ, Interferon gamma; IPA, Ingenuity pathway analysis; IVCL, *in vitro* cultivated *Leptospira*; LPHS, Leptospiral pulmonary haemorrhage syndrome; NSV, Normalised spot volume; PTM, Post translational modification; PMT, Photomultiplier tube; RC/DC, Reducing agent compatible/detergent compatible; TEMED, Tetramethylethylenediamine; TFA, Trifluoroacetic acid; Tris-HCl, Tris-hydrochloric acid.

* *Corresponding author at:* Vetsuisse Faculty University Bern, Länggassstrasse 128, 3012 Bern, Switzerland. Tel.: +41 31 631 2778; fax: +41 31 631 2275.
E-mail address: simone.schuller@vetsuisse.unibe.ch (S. Schuller).

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architecture and adhesion contributing to the dramatically increased alveolar septal wall permeability seen in LPHS.

Biological significance
The recent completion of the complete genome sequence of the guinea pig (*Cavia porcellus*) provides innovative opportunities to apply proteomic technologies to an important animal model of disease. In this study, the comparative proteomic analysis of lung tissue from experimentally infected guinea pigs with leptospiral pulmonary haemorrhage syndrome (LPHS) revealed a decrease in abundance of proteins involved in cellular architecture and adhesion, suggesting that loss or down-regulation of cytoskeletal and adhesion molecules plays an important role in the pathogenesis of LPHS. A publically available guinea pig lung proteome map was constructed to facilitate future pulmonary proteomics in this species.

1. Introduction

Leptospirosis is an important worldwide zoonotic disease affecting most mammalian species [1]. Clinical signs vary from subclinical asymptomatic infection to severe and potentially life-threatening disease. Leptospiral pulmonary haemorrhage syndrome (LPHS) is a particularly severe form of leptospirosis, which is increasingly recognized in both humans and animals and has become the major cause of death in patients with leptospirosis [2,3]. LPHS is characterized by rapidly progressive intra-alveolar haemorrhage and is associated with mortality rates between 20 and over 80% [4]. The pathogenic mechanisms of LPHS are poorly understood, hampering the development of efficient treatment strategies.

Previous studies in both animal models and human patients with LPHS have focused on pathogenic mechanisms affecting coagulation such as thrombocytopenia and disseminated intravascular coagulation [5–13], vasculitis [14–16], soluble factors derived from *Leptospira* spp [17–19] and immune mediated mechanisms [7,20,21]. So far, conclusive evidence regarding a single pathogenic mechanism of LPHS is missing. It is likely that LPHS is multi-factorial and that host as well as pathogen-related factors play a role [4].

Guinea pigs represent an important disease model for a number of infectious and non-infectious pulmonary conditions such as LPHS [7], tuberculosis [22], Legionnaires disease [23], allergic asthma [24], chronic bronchitis [25] and preterm respiratory distress syndrome [26]. Guinea pigs share a number of similarities with humans with regard to hormonal and immunologic responses [27], pulmonary physiology [28] and corticosteroid response [29] and the guinea pig immunological genes are more similar to human than mouse genes [30]. This species therefore represents a particularly important model for the human immune system. While the Broad Institute originally sequenced the guinea pig genome to 2X coverage as part of the Mammalian Genome Project to annotate the human genome, the guinea pig genome has now been published to full (7X) coverage [31]. Additionally, low sequence coverage from two outbred guinea pig strains, one additional inbred strain, and a Peruvian guinea pig as part of a SNP discovery project are currently being added [31]. These findings are freely accessible to researchers and have opened up new avenues of research investigations using genomics, transcriptomics and proteomics techniques in this species.

Several gel-free and gel-based proteomics techniques have been successfully applied to examine the dynamics of the proteome of *in vitro* grown and *in vivo* derived *Leptospira* and have demonstrated the power of both approaches [32–35]. Gel free "shot gun" techniques have been refined to allow for identification and quantification of thousands of leptospiral proteins in a single study, but rely on preformulated inclusion mass lists to direct mass spectrometry (MS) sequencing to a subset of target proteins [34]. Gel-based techniques, and in particular 2-D differential in-gel electrophoresis (DIGE), allow for a robust qualitative and quantitative analysis of more complex samples, such as infected lung tissue, including the differentiation of protein isoforms and post translationally modified proteins [36]. DIGE was therefore chosen for the present study to compare the proteomes of lung tissues from experimentally infected guinea pigs with LPHS and non-infected controls. Proteins with significant differences in abundance were identified via MS, mapped to functional groups and canonical pathways and used to generate further hypotheses with regards to potential pathogenic mechanisms of LPHS. As a secondary aim, a publically available guinea pig proteome map including 486 proteins was constructed facilitating future pulmonary proteomics in the guinea pig model [37].

2. Experimental procedures

2.1. Animal model and sample collection

All study protocols were approved by the University College Dublin Animal Research Ethics committee (Ref P-42-05; license B100/3682). Lung tissues from 6 infected and 6 non-infected weanling Hartley guinea pigs (Charles River Laboratories, UK) were used for this study. The animals were housed in individual cages containing wood shavings in an environment with a constant temperature of 18-22°C, a humidity of 50-60% and a 12 h day-night cycle. The animals were fed standard guinea pig chow and water supplemented with vitamin C *ad libitum*. After 5 days of acclimatization and health monitoring, 6 animals were infected by intraperitoneal injection of $5x10^2$ low passage *in vitro* cultivated *Leptospira interrogans* serovar Copenhageni (RJ 16441) in a final volume of 500 μl EMJH liquid culture medium. This leptospiral