Veterinary Microbiology 172 (2014) 294–300

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Clinical and haematological characterisation of *Mycoplasma suis* infections in splenectomised and non-splenectomised pigs

J. Stadler [a,1], C. Jannasch [a,1], S.L. Mack [b], S. Dietz [b], S. Zöls [a], M. Ritzmann [a], K. Hoelzle [c], L.E. Hoelzle [b,*]

[a] Clinic for Swine, Ludwig-Maximilians-University Munich, Oberschleissheim, Germany
[b] Institute of Environmental and Animal Hygiene and Veterinary Medicine (with Animal Clinic), University Hohenheim, Stuttgart, Germany
[c] Institute of Animal Nutrition, University Hohenheim, Stuttgart, Germany

ARTICLE INFO

Article history:
Received 18 February 2014
Received in revised form 27 April 2014
Accepted 4 May 2014

Keywords:
Mycoplasma suis
Haemotrophic mycoplasma
Experimental infection
Splenectomy
Haemorrhagic diathesis

ABSTRACT

Mycoplasma suis causes infectious anaemia in pigs (IAP), which can manifest in various degrees of severity depending on the virulence and the host's susceptibility. As *M. suis* cannot be cultured in vitro experimental infections of splenectomised animals play an essential role for pathogenesis research. The aim of the present study was to characterise the course of experimental infection using the highly virulent and red blood cell (RBC-) invasive *M. suis* strain KI3806, to compare the experimental course in splenectomised and non-splenectomised pigs and to correlate clinical and haematological parameters with *M. suis* blood loads. All infected splenectomised pigs ($n = 7$) were PCR-positive 2 days post infection (DPI) with maximum mean bacterial loads of 1.61×10^{10} *M. suis*/mL on 8 DPI. They developed severe anaemia and massive hypoglycaemia by 8 DPI and had to be euthanised preterm (until 8 DPI) without seroconversion. The non-splenectomised pigs ($n = 7$) became PCR-positive within 23 DPI and reached a maximum mean *M. suis* load of 1.64×10^5 *M. suis*/mL on 8 DPI. They developed mild anaemia, massive skin alterations with petechiae and haemorrhagic diathesis and seroconverted within 35 DPI.

The study demonstrated that experimental infection of splenectomised pigs with the highly virulent *M. suis* strain KI3806 induces a fulminant course of infection. In contrast, *M. suis* strain KI3806 induces a mild course of disease in non-splenectomised pigs, which resembles the situation in naturally infected pigs. Therefore, these infection models are valuable for future pathogenesis studies on acute and chronic *M. suis* infections.

1. Introduction

Mycoplasma suis is the major cause of infectious anaemia in pigs (IAP) and responsible for important economic losses in the swine industry worldwide. Due to its haemotrophic nature *M. suis* parasitises red blood cells (RBC) causing severe damage and deformation (Hoelzle, 2008; Groebel et al., 2009). The clinical outcome of IAP is strongly associated with the differences in virulence between *M. suis* strains and the susceptibility of the host. Clinical signs of acute IAP include haemolytic anaemia, high fever, icterus and haemorrhagic diathesis (Henry, 1979; Zachary and Basgall, 1985; Messick, 2004; Hoelzle, 2008; Sokoli et al., 2013). Chronic mild *M. suis* infections are reported to be connected with a variety of

* Corresponding author. Tel.: +49 711 45922427.
E-mail address: ludwig.hoelzle@uni-hohenheim.de (L.E. Hoelzle).
[1] Both authors contributed equally.

http://dx.doi.org/10.1016/j.vetmic.2014.05.012
0378-1135/

clinical syndromes comprising chronic anaemia and growth retardation in feeder pigs as well as decreased reproductive efficiency and dysgalactiae in sows (Henry, 1979; Brownback, 1981; Zinn et al., 1983; Schweighardt et al., 1986; Strait et al., 2012). Despite an intense immune response and even with antibiotic treatment, *M. suis* is able to persist in asymptomatic carrier animals for years without any detectable clinical signs. In these persistent infected animals, the acute IAP can be recurrent, e.g. during immunosuppressive events (e.g. stress, transport, and other infections) or after splenectomy (Hoelzle, 2008; Dent et al., 2013).

So far, *M. suis* cannot be cultured in vitro (Hoelzle, 2008; Schreiner et al., 2012). Therefore, experimental infections play an essential role for *M. suis* research and the current knowledge of key steps in pathogenesis has been mainly obtained from experimentally infected pigs (Zachary and Basgall, 1985; Zachary and Smith, 1985; Heinritzi et al., 1990a,b; Hoelzle et al., 2007c; Hoelzle, 2008; Groebel et al., 2009; Sokoli et al., 2013). However, characterisation of experimental *M. suis* infections were performed in the pre-PCR era and consequently quantitative data on bacterial loads during the course of infections could not be reported. Furthermore, the hitherto used splenectomised animals do not represent the physiological and immunological situation in naturally infected pigs.

Thus, the objectives of the present study were (1) to characterise the course of experimental *M. suis* infection in splenectomised and non-splenectomised pigs using the highly virulent and RBC-invasive strain KI3806 (Groebel et al., 2009; Oehlerking et al., 2011) and (2) to determine whether both, the acute and chronic form of IAP, could be simulated under experimental conditions. For this purpose, clinical and haematological parameters were correlated to *M. suis* blood loads and the humoral immune responses were monitored throughout the experiment.

2. Material and methods

2.1. M. suis strain

The highly virulent and RBC-invasive *M. suis* strain KI3806 (Groebel et al., 2009; Oehlerking et al., 2011) was maintained in splenectomised pigs by experimental infection (Hoelzle et al., 2009). For experimental infection, ethylenediaminetetraacetic acid (EDTA)-anti-coagulated blood was taken at the peak of bacteraemia (>80% of RBC infected). *M. suis* was quantified by LightCycler PCR (Hoelzle et al., 2007b) and adjusted to a final concentration of 10^8 *M. suis*/mL.

2.2. Study design

Fourteen 28-days old *M. suis*-negative piglets were included in the present study. All procedures and the experimental protocol were officially approved by the Government Office of Upper Bavaria, Munich, Germany (authorisation reference number 55.2.1.54-2532-87-12; 30.08.2012). Pigs were randomly assigned to two experimental groups (group A and B).

Table 1
Scoring system for the evaluation of the clinical conditions after experimental *M. suis* infection.

Organ/parameter	Alteration	Score
Ears	No alterations	0
	Mild cyanosis	1
	Moderate cyanosis	2
Skin	No alterations	0
	Mild pallor	1
	Moderate pallor/urticaria/petechiae	2
Mucosa	No alterations	0
	Pale	1
	Icteric	2
Body temperature	<40 °C	0
	40–42 °C	1
	>42 °C	2
Behaviour	No alterations	0
	Reduced	1
	Apathy	2
Feed intake	No alterations	0
	Reduced	1
	Anorectic	2

Group A-animals ($n = 7$) were splenectomised according to the method of Heinritzi (1984), while group B-animals ($n = 7$) were not splenectomised. All pigs from both groups were inoculated subcutaneously with 1 mL of *M. suis* containing blood (1×10^8 *M. suis*/mL blood) one week after splenectomy of group A.

A detailed clinical observation was performed daily and recorded according to a previously described scoring system with modifications (Hoelzle et al., 2009;Table 1). Samples (EDTA-anti-coagulated blood and serum) were collected 2 days before the infection and on days 2, 4, 6, 8, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77 and 83 post infection (DPI). Pigs exceeding a threshold of three scoring points per day and/or developing anaemia with packed cell volume (PCV) <25% and hypoglycaemia (blood glucose <3 mmol/L) were treated with tetracycline (40 mg/kg body weight) and glucose (25 g/L drinking water). Animals that reached at least five scoring points and developed severe anaemia (PCV <18%) and severe hypoglycaemia (1 mmol/L) were euthanised.

2.3. M. suis specific quantitative LightCycler PCR

DNA extraction and quantitative LightCycler PCR were performed as previously described (Hoelzle et al., 2007b; Ritzmann et al., 2009).

2.4. Haematological and biochemical blood analysis

Haematological parameters, i.e. RBC count, haemoglobin, PCV, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were determined using the Scil Vet ABC tool (Scil Animal Care Company GmbH). Biochemical serum parameters, i.e. glucose, iron, bilirubin, urea, creatinine, alpha amylase, and pancreas lipase were analysed using the Hitachi 911 Chemistry Analyser (Roche).