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Dose-dependent effects of *Chlamydia psittaci* infection on pulmonary gas exchange, innate immunity and acute-phase reaction in a bovine respiratory model ☆

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ABSTRACT

The respiratory pathogen *Chlamydia psittaci* naturally occurs in bovine herds and was recently shown to impair calf health in a dose-dependent manner. The aim of this study was to determine whether the functional consequences and immunological reactions of infection were dose related by quantifying the consequences of acute respiratory chlamydial infection on respiratory signs, disturbances of pulmonary gas exchange, response of the innate immune system, and acute-phase reaction. Fourteen calves were challenged intrabronchially with different *C. psittaci* doses (from 10^6 to 10^9 inclusion-forming units (ifu) per animal). Ten controls received either UV-inactivated chlamydiae or cell culture medium.

Compared to the controls, all animals challenged with live *C. psittaci* developed hypoxaemia linked to reduced haemoglobin oxygen saturation, increased alveolar–arterial oxygen partial pressure difference ($A\text{-}aO_2$) and pulmonary shunt, with symptoms following a dose-dependent pattern. Increases in lipopolysaccharide-binding protein (LBP) and leukocytes were also dose-dependent and accompanied by a regenerative left shift in neutrophil granulocytes. With the exception of LBP, which reflected the load of chlamydial cell components in the host, pathophysiological reactions were only detected in calves challenged with viable chlamydiae. These results indicate that the pathophysiological consequences of respiratory *C. psittaci* infections are strongly dependent on the challenge dose of chlamydiae. For further studies, challenge doses between 10^6 and 10^8 ifu/calf are recommended.

Introduction

Chlamydiae are Gram-negative non-motile bacteria, exhibiting an intracellular lifestyle and a unique biphasic developmental cycle. After the latest revision of taxonomy, the genus *Chlamydia* (*C.*) comprises nine species, including various human and/or animal pathogens (Kuo and Stephens, 2011). Chlamydiae are ubiquitous in cattle, and have been linked to reduced performance and a variety of production diseases (Wehrend et al., 2005; Reinhold et al., 2008a, 2011; Kemmerling et al., 2009). Within the family *Chlamydiales*, *C. pecorum* and *C. abortus* are still considered the most important potential pathogens in cattle (Markey, 2011), but *C. psittaci* has also been frequently detected in livestock (reviewed by Reinhold et al. (2011)). There is strong evidence for a role of *C. psittaci* in bovine respiratory disease (Bednarek and Niemczuk, 2005; Twomey et al., 2006), but neither the functional nor the immunological consequences of infection have yet been clarified, especially in relation to the effect of different infective doses.

The aim of this study was to characterise, using an experimental model of respiratory infection (Reinhold et al., 2012), the impact of different *C. psittaci* doses on respiratory signs, pulmonary gas exchange, innate immunity, and acute-phase reaction. To evaluate the gas exchange function of the lung and the oxygen supply of peripheral tissues, we analysed arterial blood gases and potentially altered metabolites (e.g. L-lactate) in venous blood. To assess innate immune response white blood and differential cell counts were measured, while acute phase response was assessed by measuring lipopolysaccharide-binding protein (LBP), haptoglobin (Hp) and C-reactive protein (CRP). The latter two proteins were measured on the basis that these acute phase proteins (APPs) are important in human medicine for which this infection model has clinical relevance (Reinhold et al., 2012).

☆ Part of the data was presented at the 12th International Symposium on Human Chlamydial Infections, Hof/Salzburg (Austria) 20–25 June, 2010 and the XXVI. World Buiatrics Congress, Santiago (Chile), 14–18 November, 2010.

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Materials and methods

The study was carried out in strict accordance with European and National Law for the care and use of animals. The protocol was approved by the Committee on the Ethics of Animal Experiments and the Protection of Animals of the State of Thuringia, Germany (Permit Number: 04-002/07). Animal husbandry and experiments were conducted in biosafety-level 2-areas and under supervision of the authorised Institutional Agent for Animal Protection. The number of calves enrolled in this study was the minimum required for reliable results.

Animals

Twenty-four clinically healthy calves aged 20.7 ± 2.9 days (mean ± SD) (male, Holstein–Friesian breed) were obtained from a conventional farm without a history of *Chlamydia*-associated health problems. During a quarantine period of at least 20 days (mean ± SD: 26.4 ± 4.9 days, range: 20–33 days) the animals were acclimatised to handling and group housed under standardised conditions (room climate: 18–20 °C). After the quarantine period, animals were included in the study if found to be healthy on clinical examination. Further details on health status and exclusion of co-infections have been described by Reinhold et al. (2012).

Throughout the entire study, animal husbandry was in accordance with international guidelines for animal welfare. Calves were fed individually with commercial milk replacer and coarse meal. Water and hay were supplied ad libitum. None of the feed contained antibiotics.

Study design

Four groups of calves were exposed to live *C. psittaci* (strain DC 15, described by Goellner et al., 2006; Sachse et al., 2009) and received cell culture containing 10^6 ($n = 4$), 10^7 ($n = 4$), 10^8 ($n = 4$), 10^9 ($n = 2$) inclusion-forming units (ifu) per animal suspended in 6 mL of stabilising medium SPGA (containing saccharose, phosphatile substances, glucose and bovine albumin; Bovarnick et al., 1950). The two control groups received either 6 mL medium containing UV-inactivated *C. psittaci* equivalent to 10^8 ifu ($n = 6$) or ink-spiked stabilising medium (5 mL per animal; dilution: 1:5, $n = 4$). On the day of inoculation, the age of the calves ranged from 41–54 days and their weight ranged from 64 to 87 kg (mean 71 ± 6 kg).

Preparation of the inactivated inoculum and the endoscopic administration of this inoculum into eight defined bronchi was as described by Reinhold et al. (2012). A respiratory score was used to assess both presence and severity of respiratory illness on a daily basis (Supplementary Table 1). Arterial (a) and venous blood samples were collected daily before morning feeding from 1 day before challenge until the animals were euthanased 2–3 days post inoculation (dpi). The blood sampling and analysis regime is shown in Table 1.

Catheterisation of the abdominal aorta

In each calf, the abdominal aorta was catheterised 1 day before challenge to facilitate collection of arterial blood. After shaving and disinfecting the skin of the lumbar region spaciously, calves were sedated with xylazine (Rompun 2%, Bayer, 0.05 mg/kg bodyweight) 10 min prior to catheterisation. To insert the catheter, the method of direct puncture, first described by Logvinov (1971), was modified as follows: the puncture site was determined by palpating the distal boundary of the longissimus lumborum muscle between the transverse processes of lumbar vertebrae L3 and 4.

A custom-made cannula with stylet (length 25 cm; outer diameter, 3.0 mm; inner diameter, 2.5 mm; stainless steel) was inserted parallel to the transverse processes at an angle of 30–40° to the vertebral column. To puncture the aorta the cannula was advanced medially passing the vertebral body in close proximity. Success was indicated by pulsing flow of arterial blood upon removal of the stylet. A sterile polyurethane catheter (16 G, length, 70 cm; Cavafix Certo, B. Braun) with stylet was inserted through the lumen of the cannula. The catheter stylet was removed and the distal luer-lock connection of the catheter was cut off with sterile scissors. The steel cannula was removed very slowly to avoid secondary haemorrhage.

A three-way stopcock was attached to the protruding catheter tube by inserting an 18 G needle into the catheter. To prevent clotting, the catheter was flushed and filled with heparinised 0.9% sterile saline solution (500 IU heparin/mL; Heparin–Natrium-25,000-ratiopharm, Ratiopharm) immediately after insertion and after each

Table 1
Analysis of blood samples: parameter, methods and sampling times.

Parameter	Abbreviation	Measurement device	Blood-source	Time points				
				Before inoculation		Days after inoculation		
				1 day	1 h	1	2	3
Blood gases								
(a) Parameters measured with manufacturers standard electrodes								
Partial pressure of oxygen	$p(a)O_2$	ABL 725, BT	Arterial		x	x	x	x
Partial pressure of carbon dioxide	$p(a)CO_2$	ABL 725, BT	Arterial		x	x	x	x
pH	–	ABL 725, BT	Arterial		x	x	x	x
(b) Mathematically derived parameters								
Alveolar–arterial difference for $p(a)O_2$	$A\text{-}aO_2$	ABL 725, BT	Arterial		x	x	x	x
Percentage of Hb saturated with O_2	$sO_2(a)$	ABL 725	Arterial		x	x	x	x
Pulmonary shunt	Shunt	ABL 725	Arterial		x	x	x	x
Partial pressure of oxygen at 50% Hb saturation	p50	ABL 725	Arterial		x	x	x	x
Haemoxymetry								
Haematocrit (calculated)	Hct (a)	ABL 725	Arterial		x	x	x	x
Concentration of total Hb	ctHb(a)	OSM3	Arterial		x	x	x	x
Percentage of oxy-Hb in total Hb	$O_2Hb(a)$	OSM3	Arterial		x	x	x	x
Percentage of deoxy-Hb in total Hb	HHb(a)	OSM3	Arterial		x	x	x	x
Electrolytes – measured with manufacturer's standard electrodes								
Sodium concentration	cNa^+	ABL 725	Venous	x	x	x	x	x
Potassium concentration	cK^+	ABL 725	Venous	x	x	x	x	x
Calcium concentration	cCa^+	ABL 725	Venous	x	x	x	x	x
Chloride concentration	cCl^-	ABL 725	Venous	x	x	x	x	x
Metabolites – measured with manufacturer's standard electrodes								
Glucose concentration	cGlc	ABL 725	Venous	x	x	x	x	x
L-Lactate concentration	cLac	ABL 725	Venous	x	x	x	x	x
Acute phase proteins (APPs)								
Lipopolysaccharide-binding protein	LPB	ELISA	Serum	x	x	x	x	x
Haptoglobin	Hp	ELISA	Serum	x	x	x	x	x
C-reactive protein	CRP	ELISA	Serum	x	x	x	x	x
White blood cell count								
Includes: lymphocytes, monocytes, granulocytes (neutrophils, basophils, eosinophils)	WBC	Microscopy	Venous	x	x	x	x	x

BT, corrected for individual body temperature measured immediately before blood sampling; Hb, haemoglobin.