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Original article

Dose dependency and individual variability in selected clinical, haematological and blood biochemical responses after systemic lipopolysaccharide challenge in cattle

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Abstract – Previous studies have noted that susceptibility to systemic lipopolysaccharide (LPS) exposure seems to differ between individual cows. However, to date inter-individual variation in the response to intravenous injection of LPS has been reported only as an empirical finding, and its existence or extent has never been backed up by statistical analyses. The aim of the present study was therefore to investigate the dose-dependency of clinical, haematological and blood biochemical responses to intravenous LPS injection in dairy cattle and to determine the extent to which these responses differed between individual cows. Eight dairy cows each received three intravenous injections of *Escherichia coli* LPS (10, 100, and 1000 ng/kg, consecutively) at three-week intervals. All three LPS doses induced clinical, haematological, and blood biochemical responses lasting up to several days. The strength of all of the responses increased significantly with an increasing LPS dose. A statistically significant inter-individual variation was demonstrated for all clinical, haematological, and blood biochemical responses except for serum calcium concentrations. More than half of the statistical variation in white blood cell and thrombocyte counts could be attributed to the individual. The results of this study show that despite the existence of a dose-response relationship between LPS and ensuing clinical, haematological, and blood biochemical responses, the majority of responses to LPS differ significantly in strength and duration from cow to cow.

lipopolysaccharide / susceptibility / dose-response / individual variation

1. INTRODUCTION

Lipopolysaccharide (LPS) is a structural part of the outer cell wall of Gram-negative bacteria. It is present in the gastrointestinal tract without causing disease. However, when even small amounts of LPS enter the systemic circulation of cattle (e.g. through translocation from the gastrointestinal tract or sites of Gram-negative infection) they provoke a severe clinical response dominated by signs of systemic inflammation.

LPS is implicated in several common bovine diseases, in particular in Gram-negative infections such as coliform mastitis, neonatal coliform septicaemia, lung pasteurellosis and salmonellosis. It has also been linked to the development of non-infectious

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diseases such as ruminal acidosis, laminitis and displaced abomasum [3, 8, 11]. Its potential to cause harm is particularly important in cattle, since cattle appear to be several thousand times more sensitive to LPS than common laboratory species [6]. Even low-dose exposure may therefore have a severe effect on dairy cows. Such an effect is especially pronounced in cows in early or peak lactation, since these individuals maintain a delicate physiological balance.

It has been demonstrated repeatedly that the severity of *Eschericia coli* (*E. coli*) mastitis varies considerably between cows. Factors determining susceptibility to intramammary *E. coli* inoculation have been extensively investigated (recently reviewed by [9]). The outcome of *E. coli* mastitis depends mainly on cow factors, and it has therefore been suggested that cows may be classified as either moderate or severe responders [19, 40]. It seems that the ability to resist to experimentally induced *E. coli* mastitis remains stable over short time periods, since cows challenged by intramammary inoculation of *E. coli* twice with three-week intervals had the same clinical response pattern (severe versus moderate/mild) after both challenges [20].

A number of studies report large individual variation in responses after intravenous LPS injection [1, 16, 21, 28, 35]. However, in contrast with *E. coli* mastitis, the factors underlying differences in susceptibility to systemic exposure to LPS have been investigated only sparingly in cattle. Since it has often been proven difficult to experimentally handle this individual variation, previous studies suggest that differential ability to withstand the harmful effects of LPS may exist in the bovine [30]. However, as yet, no published studies have attempted to statistically quantify individual variation in response to systemic LPS exposure in cattle.

The aim of the present study was to investigate some clinical, haematological and blood biochemical responses in adult dairy cows to an intravenous, low-dose LPS challenge. The study was designed to evaluate dose-dependent effects of LPS and the extent of inter-individual variation in the response to LPS.

2. MATERIALS AND METHODS

2.1. Animals and experimental procedures

Eight clinically healthy[1], non-pregnant, non-lactating Danish Holstein cows (cows I to VIII), of first to fifth parity (age 3 to 7 years) and weighing (mean ± standard deviation) 603 ± 59 kg were included in the study. The cows were stabled in tie stalls and fed grass silage, hay and water ad libitum for four weeks before and throughout the experiment. They were acclimatized to all procedures prior to experimentation. The procedures were approved by the Danish Animal Experimentation Inspectorate.

One week before each challenge, the cows were equipped with indwelling venous catheters (Secalon® Seldy, Becton Dickinson, Brøndby, Denmark). These were inserted into one of the larger ear veins (v. auricularis intermedia or v. auricularis medialis) and passed through the v. auricularis caudalis into the v. jugularis externa. At each blood sampling the cows' ears were examined for inflammatory reactions and catheters were flushed with approximately 15 mL 0.9% sterile saline. To prevent clotting, 1 mL 0.9% sterile saline containing 50 IU heparin (Løvens Kemiske Fabrik, Ballerup, Denmark) was deposited in the catheter. Prior to flushing or blood collection, approximately

[1] At the beginning of the acclimatization period (four weeks before the experiment) clinical examinations of the udder, uterus and limbs were performed, and cows IV and VII were subsequently treated with intramammary antibiotics. During the experimental period clinical examinations of the udder, limbs and ears were performed daily. Mild periphlebitis of the auricular vein was diagnosed in cow VII at 96, 120 and 144 h of the 10 ng LPS/kg challenge. This was treated topically and healed within a week. Cow IV was treated for Gram-positive mastitis at 120 and 144 h of the last challenge.

5 mL blood and heparin were aspirated and discarded.

At three-week intervals the cows were challenged by intravenous injection of rising doses of *E. coli* LPS (10, 100, and 1000 ng LPS/kg, consecutively; *E. coli* O55:B5, Westphal extraction; Sigma Chemical Company, St. Louis, USA). The increase in doses and the interval between injections were chosen to minimize the effects, if any, of LPS tolerance. In each challenge, LPS was injected intravenously at 0 h through the indwelling venous catheter. The cows were examined clinically and blood was sampled at –144, –120, –96, –72, 0, 0.5, 1, 2, 3, 4, 5, 6, 12, 24, 36, 48, 72, 96, 120, and 144 h relative to injection. Clinical examination involved the recording of rectal temperature, heart rate, respiratory rate and ruminal motility. Blood was collected in 30-mL single-use syringes (B. Braun Melsungen, Melsungen, Germany). It was immediately transferred either to tubes (Vacutainer System, Becton Dickinson, Brøndby, Denmark) containing sodium-EDTA for determination of white blood cell count (WBC) and thrombocyte count, or to tubes with no additive for the analysis of serum calcium, zinc and iron concentrations. Serum samples were prepared by letting the blood samples clot at room temperature before being centrifuged at 2500 *g* for 15 min at 4 °C. Serum samples were stored at –18 °C until analysis.

2.2. Laboratory analyses

WBC and thrombocyte counts were made with an automatic cell counter (CELL DYN 3500, Abbot Laboratories A/S, Gentofte, Denmark). Serum concentrations of calcium and zinc were assessed by atomic absorption spectrophotometry (AAS 5000, Perkin Elmer, Allerød, Denmark) and serum concentrations of iron were determined by colometric spectrophotometry (ADVIA 1650, Bayer A/S, Lyngby, Denmark). In each challenge, iron and zinc were determined at –144, 0, 3, 6, 12, 24, 36, 48, 72, 96, 120, and 144 h, and calcium was determined at –144, 0, 1, 2, 4, 6, 12, 24, 36, 48, 72, and 96 h.

2.3. Statistical analyses

Statistical analysis was performed as a repeated measurement analysis of variance (ANOVA) using the PROC MIXED procedure of Statistic Analytical Software (version 8, SAS® Institute, Cary, North Carolina, USA). The following explanatory variables were included in the model as fixed effects: LPS dose (dose), time in hours after challenge (hour), cow identity (cow ID) and the interaction between LPS dose and time (dose × hour). The outcome variables were rectal temperature, heart rate, respiratory rate, ruminal motility, WBC, thrombocyte count, and serum calcium, zinc and iron concentrations. The correlation structure between repeated measurements for each cow within each dose was modelled by a spatial power structure. Assumptions were checked on residual plots and tested for normality. Unless otherwise stated, a 5% level of significance was used.

Differences in least squares means estimates identified through the repeated measurement analyses were used to determine significant increases from baseline values and time intervals where the respective responses to 10, 100, and 1000 ng LPS/kg differed significantly. The Bonferroni multiple comparison procedure was used to control Type I errors.

Differences in residuals between the full statistical model described above and a model in which the variable cow ID was omitted were used to assess the extent to which the variation in the outcome variables was explained by the individual cow.

The time needed to re-establish baseline rectal temperature and ruminal motility was determined for each cow during the 1000 ng LPS/kg challenge. Individual 95% confidence intervals were calculated for the mean of pre-challenge values, and the time needed for the response to return to a value

within the limits of the confidence interval was determined for each cow.

Figures 1, 2 and 3 were prepared using least squares means estimates obtained in the statistical analysis. Figure 4 was based on raw data.

3. RESULTS

3.1. LPS-induced responses

All three LPS doses induced significant clinical signs as well as haematological and blood biochemical responses in all cows (Tab. I). The cows developed depression, shivering, salivation, miosis, anorexia, hyperthermia (Fig. 1A), tachycardia (Fig. 1B), tachypnea (Fig. 1C), ruminal stasis (Fig. 1D), diarrhea, leukopaenia followed by leukocytosis (Fig. 2A), thrombocytopaenia (Fig. 2B), hypocalcaemia (Fig. 3A), hypozincaemia followed by hyperzincaemia (Fig. 3B), and hypoferraemia (Fig. 3C). Clinical signs commenced within 30 min following LPS injection. Many of the responses persisted for several days, particularly after challenge with 100 and 1000 ng LPS/kg (Tab. I).

Rectal temperature peaked in a mono-, bi-, and triphasic pattern in challenges with 10, 100, and 1000 ng LPS/kg, respectively (Fig. 1A, Tab. I). Temperature peak values were significantly lower in 100 and 1000 ng LPS/kg challenges than in the 10 ng LPS/kg challenge (Tab. II). Least squares means estimates of maximum peak values were 40.0, 39.5 and 39.0 °C, and times to maximum peak after challenge were 5, 6 and 12 h in the 10, 100 and 1000 ng LPS/kg challenges, respectively.

The duration of ruminal hypomotility increased with dose. It averaged 5 h for the 10 ng LPS/kg challenge, 24 h for the 100 ng LPS/kg challenge and 36 h for the 1000 ng LPS/kg challenge (Fig. 1D, Tab. I).

Leukopaenia, but no significant leukocytosis, developed in response to the challenge with 10 ng LPS/kg. Significant leukopaenia followed by significant leukocytosis developed after challenge with the two highest LPS doses (Fig. 2A, Tab. I). Serum levels of calcium, iron and zinc decreased significantly in all three challenges. In the challenges with 100 and 1000 ng LPS/kg the cows developed hyperzincaemia within 72 to 120 h of injection of LPS. The hyperzincaemia persisted throughout the study period (Fig. 3, Tab. I).

3.2. Dose-dependency of LPS-induced responses

Repeated ANOVA measurements (Tab. III) showed that all responses were dose-dependent. Moreover, the effects of dose and time were interdependent for all responses except serum calcium concentrations, showing that the dose-dependency changed over the course of the challenges. Time points, or intervals, at which responses to the three challenges differed, are summarized in Table II. Hyperthermia, tachypnea, ruminal hypomotility and leukopaenia/leukocytosis intensified with each increase in LPS dose. Tachycardia, thrombocytopaenia and alterations in serum concentrations of calcium and zinc intensified mainly between the two first challenges with only a small additional intensification after the challenge with 1000 ng LPS/kg. Serum concentrations of iron were significantly different only between the first and the second challenge. Increasing the LPS dose to 1000 ng/kg did not result in more pronounced hypoferraemia.

3.3. Individual variation in LPS-induced responses

All of the clinical signs, and all of the haematological and blood biochemical changes except serum calcium concentrations, differed significantly in strength between cows (Tab. III). The variation that could be attributed to the individuals ranged from 7.7 to 60%. Especially with regards to WBC and thrombocyte counts, explanatory variable cow ID accounted for a large part of the statistical variation (Tab. III).

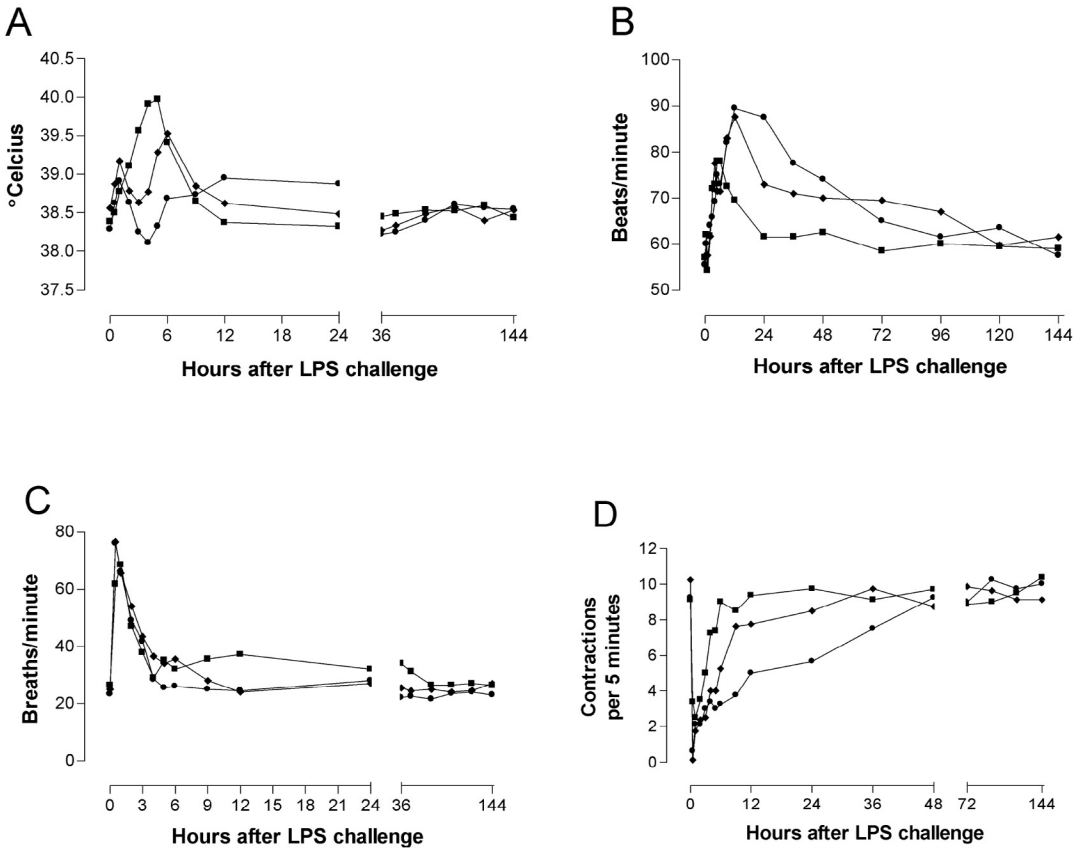

Figure 1. Average least squares means estimates of rectal temperature (A), heart rate (B), respiratory frequency (C), and ruminal motility (D) after intravenous injection of 10 (■), 100 (♦), and 1000 (●) ng *Escherichia coli* lipopolysaccharide/kg. Values that deviate significantly from the baseline in the three challenges are summarized in Table I. Values that differ significantly between challenges are summarized in Table II.

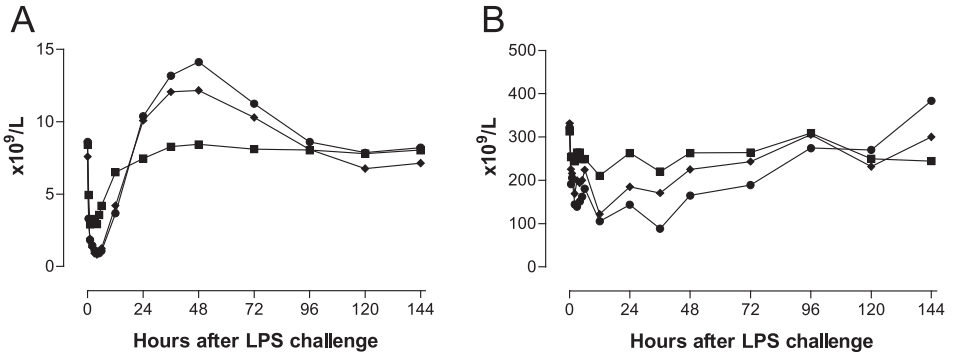

Figure 2. Average least squares means estimates of white blood cell count (A) and thrombocyte count (B) after intravenous injection of 10 (■), 100 (♦), and 1000 (●) ng *Escherichia coli* lipopolysaccharide/kg. Values that deviate significantly from the baseline in the three challenges are summarized in Table I. Values that differ significantly between challenges are summarized in Table II.

The duration of responses also varied markedly between individuals. For example, after challenge with 1000 ng LPS/kg the time needed to re-establish ruminal motility ranged from 4 to 96 h (Fig. 4) and the time needed for rectal temperature to return to the baseline ranged from 30 min to 36 h (data not shown).

4. DISCUSSION

4.1. LPS-induced responses

The responses to systemic exposure to LPS demonstrated in this study were similar to those reported earlier [7, 13, 27, 37]. All three LPS doses used in the present study induced obvious clinical signs in all cows. Although previous studies have shown that LPS doses in this range result in increased serum concentrations of arachidonic acid metabolites [2, 12], this was the first study to show clinical signs arising from LPS doses as low as 10 ng/kg. Only minute LPS concentrations have been detected in the systemic circulation of cattle with naturally occurring coliform mastitis and experimentally induced ruminal acidosis [4, 10]. Low-dose experimental LPS challenges may therefore elicit clinical, haematological and blood-biochemical responses that mimic the pathophysiology of naturally occurring LPS-mediated diseases.

The bolus injection of LPS employed in this study induced responses that lasted for several hours or days. During naturally occurring LPS-associated diseases, LPS probably translocates from the gastrointestinal tract or the site of Gram-negative infection repeatedly or continuously for some time. When this happens the cow may experience anorexia, depression, hyperthermia, and ruminal hypomotility for an extended period of time.

The inverse relationship between rectal temperature and LPS dose demonstrated in this study has been reported previously [27]. Moreover, very moderate or absent pyrogenic responses to LPS reported in ruminants [16, 23, 31] have led to the suggestion that fever is not the most suitable parameter for monitoring LPS effects [29]. The temperature response to LPS is very complex, and the pyrogenic effect of LPS may be obscured as changes in metabolism and blood circulation become greater with increasing doses of LPS. The observed mono-, bi- and triphasic febrile responses to 10, 100, and 1000 ng LPS/kg, respectively, were also consistent with what has been shown previously [7, 13, 37]. The mechanisms underlying the multiple peaks here are not

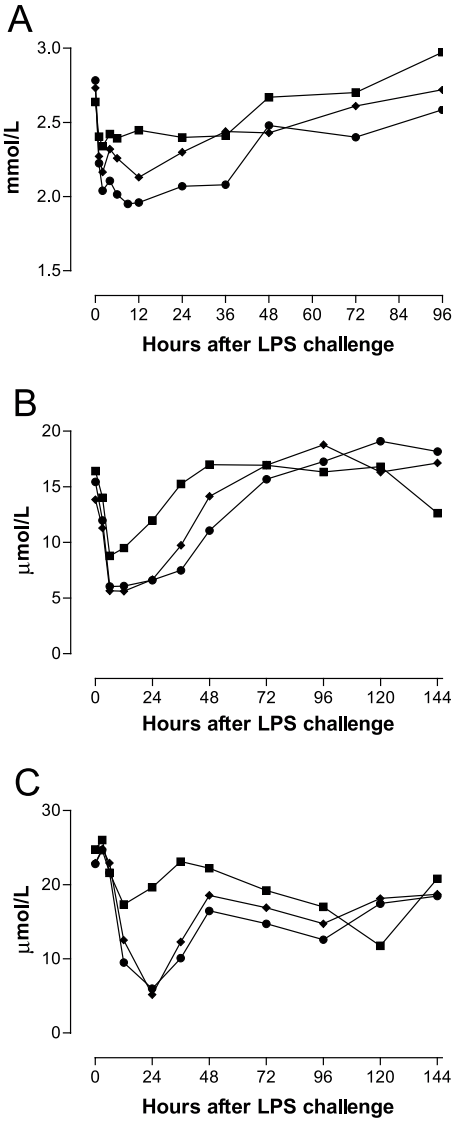

Figure 3. Average least squares means estimates of serum calcium (A), serum zinc (B) and serum iron (C) concentrations after intravenous injection of 10 (■), 100 (♦), and 1000 (●) ng *Escherichia coli* lipopolysaccharide/kg. Values that deviate significantly from the baseline in the three challenges are summarized in Table I. Values that differ significantly between challenges are summarized in Table II.

fully understood. It has been suggested that the first peak is mediated by the early release of prostaglandin E_2 within the brain, and that subsequent peaks are mediated by interleukin-1 induced prostaglandin E_2 production [27].

Redistribution of iron and zinc within the body after LPS injection causing decreased serum levels of the microminerals has been observed repeatedly in ruminants [18, 25, 28, 38]. Most studies cease data collection within hours or a few days of LPS injection, which may explain why the present study was the first to demonstrate hyperzincaemia in the late phase of LPS response. A recent study of experimental endotoxin injection in buffalo calves showed hypozincaemia but failed to demonstrate hyperzincaemia, although the animals were observed for 120 h after the challenge [21]. The causes of this discrepancy are not known, but they may be related to differences between breeds or LPS types.

4.2. Dose-dependency of LPS-induced responses

The clinical, haematological, and blood biochemical responses assessed in this study became stronger or lasted longer with increased LPS doses. This was consistent with findings in calves in other studies [15, 28]. However, not all the responses were equally dose-responsive. Some intensified with each increase in LPS dose. Others intensified mainly between the first and the second challenge.

Several explanations of this are available. First, the numerous hormones and inflammatory mediators (e.g. cytokines and arachidonic acids) that cause the clinical, haematological and blood biochemical changes in response to LPS [12, 33, 39] may not all be synthesized in a dose-dependent manner. For example, plasma concentrations of pro-inflammatory cytokines did not increase in a straightforwardly dose-dependent manner in calves challenged with increasing amounts of LPS [14].

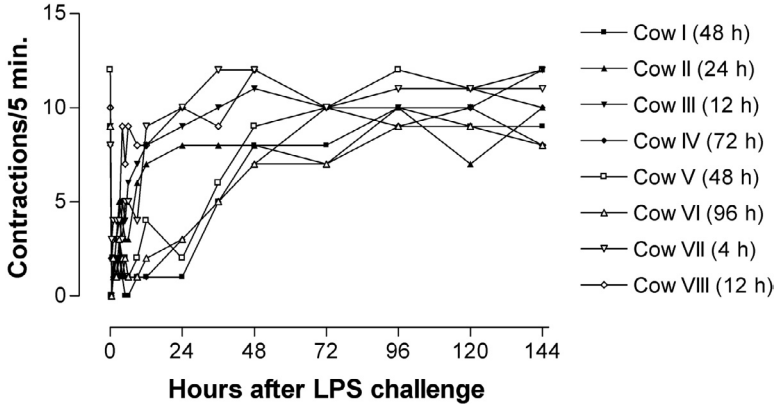

Figure 4. Changes in ruminal motility in individual cows after challenge of 1000 ng *Escherichia coli* lipopolysaccharide/kg. Numbers in parentheses are the time needed to re-establish baseline ruminal motility.

Table I. Duration of the alterations (decrease and/or increase) in the clinical, haematological, and blood biochemical responses to lipopolysaccharide (LPS)[a].

Response parameter	10 ng LPS/kg	100 ng LPS/kg	1000 ng LPS/kg
Rectal temperature	1–6	0.5–1, 5–6	0.5–2, 6–24, 96
Heart rate	3–12	3–72	4–48
Respiratory rate	0.5–3, 5, 9–12, 36	0.5–6	0.5–3
Ruminal frequency	0.5–5	0.5–24	0.5–36
White blood cell count	0.5–12	0.5–72	0.5–72
Thrombocyte counts	0.5–2, 12, 36	0.5–72, 120	0.5–72
Calcium	1–2, 96	1–24	1–72
Zinc	3–24, 144	3–36, 72–96, 144	3–48, 120–144
Iron	12, 96–120	12–36, 72–96	12–96

[a] Numbers are time intervals (in hours) where the responses to 10, 100, and 1000 ng LPS/kg were significantly different from baseline levels ($P < 0.05$). In each of the three challenges *Escherichia coli* LPS was injected intravenously at 0 h. Refer to Figures 1 to 3 for a comparison with the course of the responses in the three challenges.

Secondly, inflammatory responses to LPS prompt several anti-inflammatory mechanisms whose purpose is to restore homeostasis. This is necessary because the response – although crucial for controlling infections with Gram-negative bacteria – is capable of inducing pathophysiological changes in the host if it reacts excessively. Such feedback mechanisms may cause certain responses to have a dose-response relationship with LPS only in a rather narrow range of doses.

Thirdly, it has been recognized for decades that a state of transient clinical hyporesponsiveness known as "LPS tolerance" is induced by repeated exposure to LPS [5, 22]. The effects of this condition in ruminants include marked reduction in strength and/or duration of LPS responses. This

Table II. Dose-dependency of the responses to lipopolysaccharide (LPS)[a].

Response parameter	10 vs. 100 ng LPS/kg	100 vs. 1000 ng LPS/kg	10 vs. 1000 ng LPS/kg
Rectal temperature	–144, 0.5–5	–144, 3–6, 12–24	2–6, 12–24
Heart rate	9–24, 72	24	12–48
Respiratory frequency	0.5, 12, 36	4–6	–96, 0.5, 5, 9–12, 36–48
Ruminal motility	0.5, 3–6	6–36	0.5, 3–24
White blood cell counts	2–72	48	2–72
Thrombocyte counts	12–24	36, 144	2–5, 12–48, 144
Serum calcium	12	36	4–36, 72–96
Serum zinc	0–48, 144	48, 120	6–48, 144
Serum iron	–144, 24–36, 120	–	–144, 12–48, 120

[a] Numbers are time intervals (in hours) after injection where the responses to 10, 100, and 1000 ng LPS/kg differed significantly ($P < 0.05$) in strength. In each of the three challenges, *Escherichia coli* LPS was injected intravenously at 0 h. Refer to Figure 1 to 3 for comparison with the course of the responses in the three challenges.

Table III. Significance of effects of time, lipopolysaccharide (LPS) dose, individual, and the interaction between time and LPS dose on clinical, haematological, and blood biochemical responses to LPS; and percentage of variation accounted for by the individual.

Response parameter	*P*-values				Variation accounted for by the individual[a] (%)
	Time (hour)	LPS dose	Individual	Time × dose	
Rectal temperature	< 0.001	< 0.001	0.0048	< 0.001	7.7
Heart rate	< 0.001	0.0197	< 0.001	0.0084	36
Respiratory frequency	< 0.001	0.0024	< 0.001	< 0.001	26
Ruminal motility	< 0.001	< 0.001	< 0.001	< 0.001	12
White blood cell counts	< 0.001	0.1390	< 0.001	< 0.001	60
Thrombocyte counts	< 0.001	0.0053	< 0.001	0.0058	60
Serum calcium	< 0.001	< 0.001	NS	NS	–
Serum zinc	< 0.001	0.0155	0.0030	< 0.001	32
Serum iron	< 0.001	0.0022	< 0.001	< 0.001	35

[a] Calculated as differences in residuals between the full statistical model (time, LPS dose, individual and interaction between time and LPS dose as explanatory variables) and a statistical model in which the individual was left out.
NS = not significant.

weakening of response has in most cases been achieved by injecting LPS one or more times daily, or even hourly, often for several consecutive days [35–37]. Where injections have been less frequent, the weakening was less pronounced [7, 13]. Tolerance is complex and its pathogenesis imperfectly understood. However, temporary inhibition of the synthesis of pro-inflammatory cytokines and the development of anti-LPS antibodies are believed to underlie the early and late phases of clinical tolerance, respectively. Induction of early-phase tolerance is proportional to the amount of LPS injected [17]. In order to accommodate for this, the LPS doses were sequentially increased in the present study. Cytokine synthesis is restored a few hours or days after LPS exposure [22,

24, 26, 34]. Given this, and given that the LPS injections involved in the present study were three weeks apart, early-phase tolerance probably had little or no effect on our results. The LPS used in this study was prepared by Westphal extraction. This method delivers a highly purified compound with poor antigenicity, which induces no detectable antibodies and therefore minimal late-phase tolerance [17]. In view of these facts, and because several of the clinical, haematological, and blood biochemical responses assessed in the present study showed unambiguous dose-response relationships, it is unlikely that LPS tolerance, as the phenomenon is currently understood, influenced the responses to LPS in the model of repeated challenges reported in this paper.

4.3. Individual variation in LPS-induced responses

The experimental design in the present study allowed systematic evaluation of individual variability in responses to intravenously injected LPS. Several previous studies have reported wide inter-individual variation in susceptibility of cattle to systemic LPS challenge as an empirical finding [1, 16, 21, 28, 35], but to our knowledge this was the first attempt to statistically quantify the individual differences.

Responses to LPS are not caused directly by the toxicity of LPS. They are induced by inflammatory mediators released from activated host cells. The hypothesis of the present study, based on findings in studies on *E. coli* mastitis [9], was that inter-individual differences in the orchestration of the inflammatory response substantially influence the strength and duration of dose-dependent responses to intravenously injected LPS. Hence it seems probable that both LPS-induced responses of individual cows will differ considerably and that this variation will admit of statistical quantification (i.e. that there will be a statistically significant and systematic effect of the cow). The results of the present study demonstrate that all clinical, haematological, and blood-biochemical parameters except serum calcium concentrations do indeed display statistically significant inter-individual variation.

The pathogenesis of the inter-individual variation in LPS susceptibility was not investigated. Others have suggested that factors influencing the inflammatory response – e.g. levels of antibodies against lipid A, capacity for synthesis of tumour necrosis factor α, and the genetically determined number of LPS receptors on macrophages – may contribute to resistance to the effects of LPS [30, 32, 35].

Susceptibility to LPS may change over time, with changes in physiological status or age. In the present study, the cows had a similar physiological status (non-pregnant, non-lactating). There were considerable differences in age, but the statistical analysis showed no significant effect of this parameter on the responses to LPS (data not shown). Whether the observed inter-individual variation is "idiosyncratic" (i.e. a peculiarity of physical constitution or a characteristic belonging to – and distinguishing – an individual), and hence whether a given animal has a consistent ability to cope with the effects of intravenously injected LPS, remains to be determined.

Leukocytic and thrombocytic responses showed the greatest individual variation: more than half of the statistical variation in them was attributable to the individual. This variation may cause some of the individual variation in the clinical and blood biochemical parameters, because LPS exerts its effects through various mediators released by activated cells, including leukocytes and thrombocytes. The reactivity of leukocytes and thrombocytes and the orchestration of the inflammatory response in the individual cow may thus determine a cow's clinical response to LPS.

Owing to the diversity of clinical, haematological and blood-biochemical responses to systemic LPS exposure, it was not possible to classify cows as moderate or severe responders in the present study.

In conclusion, the present study shows that responses to systemic LPS challenge (i) are elicited by extremely low doses of LPS, (ii) are dose-dependent, and (iii) differ significantly in strength and duration between individual cows. This inter-individual variation may explain important differences in the ability of cows to resist and tolerate an inflammatory insult. This study thus emphasizes the importance of taking individual factors into account when working with LPS-associated diseases. Elucidation of the pathogenesis underlying inter-individual variation in LPS-induced responses may improve our understanding of the pathophysiology of LPS-associated diseases in cattle.

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