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Behavioral and patho-physiological response as possible signs of pain in dairy cows during *Escherichia coli* mastitis: A pilot study

Alice de Boyer des Roches,*[1] Marion Faure,* Alexandra Lussert,† Vincent Herry,† Pascal Rainard,‡ Denys Durand,* and Gilles Foucras†
*Université Clermont Auvergne, Institut National de la Recherche Agronomique (INRA), VetAgro Sup, UMR Herbivores, F-63122 Saint-Genès-Champanelle, France
†Université de Toulouse, Ecole Nationale Vétérinaire de Toulouse (ENVT), INRA, Interactions Hôtes-Agents Pathogènes (IHAP), F-31076 Toulouse, France
‡Infectiologie et Santé Publique (ISP), INRA, Université de Tours, UMR1282, F-37380 Nouzilly, France

ABSTRACT

Bovine mastitis is one of the most common diseases in the dairy industry and it is a major welfare problem. Pain during mastitis is generally assessed through behavior but a combination of indicators would increase the chances of detecting pain and assessing its intensity. The aim of this study was to assess behavioral and patho-physiological responses as possible signs of pain experienced by cows after experimental intramammary challenge (mastitis) with *Escherichia coli*. Six Holstein-Friesian cows received an inoculation of *E. coli* P4 in one healthy quarter. Evolution of the disease was assessed using bacteriological growth and somatic cell counts (SCC). Cows' response to the challenge was monitored by direct behavioral and clinical observations, data loggers, rumen temperature sensors, and indicators of inflammation, stress, and oxidative status. From all data recorded, the variables that contributed most to the discrimination of mastitis phases were obtained by factorial discriminant analysis. Baseline levels of all indicators corresponded to values before challenge. Specifically, we weighted data relating to lying behavior by the observations at the same hour of the day before challenge to eliminate the circadian rhythm effect. We identified 3 phases that were discriminated by factorial discriminant analysis with good performance. Nine indicators varied according to the phase of the disease: cows' attitude toward their surroundings, tail position, clinical signs, ear position, variation of postural changes, concentrations of haptoglobin and serum amyloid A (SAA), cortisol blood levels, and rumen temperature (as a surrogate for body temperature). In phase 1 (4 to 8 h postinoculation), *E. coli* proliferated exponentially in milk but inflammation indicators remained at baseline levels. Cows were less attentive toward their surroundings (median score, 0.63), and postural changes (lying/standing) were less frequent (0.75 times from baseline). In phase 2 (12 to 24 h postinoculation), bacterial concentrations peaked around 12 h and then began to decrease concomitantly with a sharp SCC increase. Cows were less attentive toward their surroundings (score, 0.54), had high plasma cortisol (31.3 ng/mL) and SAA (100.3 μg/mL) concentrations, and rumen temperature was increased (40.3°C). In phase 3 (32 to 80 h postinoculation), bacterial concentrations decreased concomitantly with high SCC levels. Cows had high levels of haptoglobin (0.57 mg/mL) and SAA (269 μg/mL) but showed no behavioral changes. Dairy cows displayed changes of behavioral, inflammatory, and stress parameters after *E. coli* mammary inoculation. Our results suggest that cows may have experienced discomfort in the preclinical phase (phase 1) and pain in the acute phase (phase 2) but neither discomfort nor pain in the remission phase (phase 3). Although larger controlled studies are needed to confirm our findings, this knowledge could be useful for early detection of *E. coli* mastitis and for decision-making regarding the initiation of pain-relief treatment during mastitis in dairy cows. This would improve animal welfare and potentially faster disease remission.

Key words: dairy cow, mastitis, pain, multiparametric analysis, *Escherichia coli*

INTRODUCTION

Mastitis is one of the major problems affecting the welfare of dairy cows (European Food Safety Authority, 2009; Leslie and Petersson-Wolfe, 2012). During mastitis, the concomitant inflammation of the udder, increased intramammary pressure, and increased external pressure (e.g., from an adjacent limb on a swollen udder) are believed to induce pain (Fitzpatrick et al., 2000; Leslie and Petersson-Wolfe, 2012). Recently,

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[1]Corresponding author: alice.deboyerdesroches@vetagro-sup.fr

cattle practitioners in the United Kingdom scored pain associated with acute *Escherichia coli* mastitis at 7 on a value scale from 0 to 10 (Huxley and Whay, 2006). Unfortunately, the management of painful conditions in cattle is still infrequently considered in practice (Hudson et al., 2008), although treating the pain experienced by cows during clinical mastitis results in decreased edema, a lower SCC, and a reduced risk of culling (McDougall et al., 2009; Fitzpatrick et al., 2013). One reason for the inconsistencies of pain relief in cattle is the difficulty in assessing pain properly (Huxley and Whay, 2006; Flecknell, 2008).

Numerous methodologies have been used to assess or quantify the levels of pain experienced by farm animals (Prunier et al., 2013). Studies on pain mainly measure an animal's pain response to a noxious stimulus by recording the incidence of a clearly defined pattern of behaviors (e.g., abnormal standing or lying; Fogsgaard et al., 2012, 2015), changes in the levels of stress response [e.g., hypothalamus-pituitary-adrenal (**HPA**) axis such as cortisol release (Hopster et al., 1998) or autonomous nervous system such as heart-rate increase (Fitzpatrick et al., 2000)], or in levels of biochemical markers of oxidative stress (Salvemini et al., 2011; Sharma et al., 2016) and inflammation (e.g., haptoglobin and serum amyloid A, **SAA**; Eckersall et al., 2001). Unfortunately, it is not possible to allocate a "pain score" based on changes in only one of these indicators because most of them are not specific or sensitive enough. For instance, HPA axis and autonomous nervous system activity are related to stressful situations (Molony and Kent, 1997; Prunier et al., 2013). Changes in activity may result from pain (Theurer et al., 2012) but can also be early signs of sickness (Veissier et al., 1989). Previous studies on how cows feel pain during mastitis generally focused on 1 or 2 types of indicators (e.g., clinical and behavioral; Fogsgaard et al., 2012), but did not, to our knowledge, combine information of various types of indicators. Such a combination would increase the chance of detecting pain and assessing its intensity. Moreover, although the severity of mastitis is well described in the scientific literature (Schukken et al., 2011), knowledge is still lacking on the levels of pain experienced by cows according to the phase of the disease (e.g., preclinical, acute phase, and remission).

The aim of this study was to assess the behavioral and patho-physiological responses, as possible signs of pain experienced by cows during *E. coli* mastitis. We hypothesized that (1) the pain experienced by cows varies according to the timeline of *E. coli* mastitis, and (2) a multiparametric approach combining several indicators, already tested in experimental surgery conditions (Faure et al., 2017), would be efficient in discriminating pain levels during mastitis.

MATERIALS AND METHODS

This experiment was carried out with the approval of the Val de Loire Ethics Committee for Experiments on Animals (France; DGRI's agreement APAFIS#813–2015061109103810v2). Animal studies were compliant with all applicable provisions established by the European Directive 2010/63/EU.

Animals, Housing, and Feeding

The study was conducted at the INRA animal facility (PFIE, Nouzilly, France). Six Holstein-Friesian cows in their first parity were used. They were part of a larger study on the effect of local immunization on the response of dairy cows to *E. coli* mastitis (Herry et al., 2017). The 6 cows used in the current study were involved as a control group in the above-mentioned study that used 18 animals in total. Detailed information on the protocol can be found elsewhere (Herry et al., 2017). Here, only information relative to the 6 animals used in the current study is provided.

The 6 cows were housed in a loose housing, deep-bedded barn (space allowance per cow, 20 m^2: 15 m^2 of bedded area and 5 m^2 of walking area) at INRA PFIE. They were fed once per day (at 1000 h) a diet based on corn silage, hay, soybean meal, and concentrate, which met the dietary requirements for transition and early lactation. The mixed ration was regularly pushed back toward the cows during the day and refusals were always >5%. They were allowed water ad libitum. The cows were milked twice a day (at 0800 and 1600 h) by experienced stockpersons in a milking parlor adjacent to the barn.

Experimental Design

The experiment was a longitudinal study, with the individual dairy cow being her own control, examining the effects of experimental *E. coli* infection. The *E. coli* strain P4 classified as O32:H37, ECOR Phylogenetic group A, and multilocus sequence type ST10 (Blum et al., 2012) was used for intramammary challenge as previously indicated.

Cows were challenged at 44 to 56 (average 49) DIM. Before challenge, all quarters were checked for the absence of IMI by performing bacteriological analysis and SCC measurement on foremilk stripping. Inoculated quarters were free of infection: the milk had SCC <50,000 cells/mL and contained no viable bacteria. One quarter of each cow was challenged by infusion with 1 mL of the bacterial suspension (1,000 cfu/mL). Inoculation was performed at midnight on d 0 just after complete milking of the gland and 8 h before the next