Veterinary Immunology and Immunopathology 172 (2016) 9–13

Contents lists available at ScienceDirect

Veterinary Immunology and Immunopathology

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

Short communication

Innate immune responses of young bulls to a novel environment

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ARTICLE INFO

Article history:
Received 23 October 2015
Received in revised form 29 January 2016
Accepted 19 February 2016

Keywords:
Young bulls
Innate immunity
Stress
Acute phase proteins
Cytokines

ABSTRACT

Animal welfare during transportation has been investigated in several studies, as opposed to post-transportation phases. In this study, we evaluated the effect of a novel environment after transportation on 26 Friesian bulls, 242 ± 42 day-old, from ten different dairy farms. Animals were shipped to a breeding center in different seasons, and selected parameters of innate immunity (serum bactericidal activity, hemolytic complement, serum albumin, α, β, and γ-globulins, interleukin-6, TNF-α) were monitored before and after the arrival at days—4/0/4/15/30. Our results showed significant differences of IL-6 and TNF-α protein levels at destination in December (94 ± 1.3 pg/ml) and June (+788 pg/ml), respectively. Moreover, the serum levels of these cytokines increased between days 0 and 15 after the arrival, the modulation of IL-6 being in agreement with established models of physical and/or psychological stress. Concerning the modulation of albumin, alpha and beta-globulins, the highest levels were detected in April, whereas a significant decrease was observed between day 15 and 30 after arrival; on the contrary, γ-globulin levels significantly increased after day 15. The results of this study highlight the occurrence of innate immune responses of young bulls to the combined effects of climate (season) and novel farming conditions.

1. Introduction

Over the last twenty years the attention of legislators for animal welfare has undoubtedly increased; in particular, several regulations have addressed farm animal welfare in distinct phases (farming, transport, slaughter). This prompted the scientific community to investigate the actual impact of these rules on animal welfare. Several studies dealt with transportation stress, focusing in particular on crucial issues like commingling, loading, time spent on trucks, exposure to extreme temperature and humidity conditions, time spent at lairage, feeding and watering during transportation (Gupta et al., 2007; Cafazzo et al., 2012; Magnani et al., 2014; Vitali et al., 2014). Remarkably, most studies neglected the post-transportation phases, i.e., the possible impact of a novel environment, new commingling, feeding and hierarchy conditions. This is a point of major importance, since integrated welfare-friendly practices should cover the whole life of farm animals, including the herd's new conditions at destination. To this purpose, the innate immune response can conveniently and efficiently depict the relevant coping efforts of farm animals. In fact, the innate immune system is able to respond to both microbial agents (infectious stressors), as well as to non-infectious stressors (high/low temperatures, endocrine disrupters, oxidative stress, hypoxia, psychological stress, tissue damage, obesity, etc.,). The common molecular basis of such responses to infectious and non-infectious stressors is represented by products related to tissue damage (Damage-Associated Molecular Patterns, DAMPs) such as salts (extracellular potassium, sodium ureate), ATP, Reactive Oxygen Metabolites (ROMs), mitochondrial DNA (Gallo and Gallucci, 2013), and by a variety of stress antigens expressed on the cell surface (Hayday, 2009). This is the reason why we evaluated in our study the effect of a novel envi-

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http://dx.doi.org/10.1016/j.vetimm.2016.02.014
0165-2427/

ronment and transportation in terms of innate immune responses of young bulls.

2. Materials and methods

2.1. Animals

The study was carried out on 26 Friesian bulls, 242 ± 42 day old, selected from different dairy farms. Some of them were born to the same sire within their farm. Animals were divided into 5 groups and transferred to a quarantine pen at the genetic test station of the National Association of Holstein Breeders of Italy (ANAFI) in different seasons. The shipment time varied between 2 and 4 h. Animals of group 1 (4 animals, 2 farms) were transferred and housed in November–December, group 2 (7 animals, 3 farms) in January–February, group 3 (6 animals, 4 farms) in April–May, group 4 (4 animals, 1 farm) in May–July and group 5 (5 animals, 4 farms) in July–August (Table 1). All animals, before the introduction into the ANAFI center, were submitted to the following checks: clinical examination, skin test for bovine tuberculosis, and serological tests for antibodies to *Brucella* spp., Blue Tongue (ELISA, OIE Manual for Terrestrial Animals 2014 cap 2.1.3 par B.2.3), Enzootic Bovine Leucosis (AGID, DPR n°230 01/03/1992 SO GU n°66 19/03/1992 All G cap II-A and DM n°358 02/05/1996 GU n°160 10/07/1996) and Infectious Bovine Rhinotracheitis viruses (ELISA, OIE Manual for Terrestrial Animals 2010 cap 2.4.13 par B.2.b). Four days after the entry into the center, all bull calves were vaccinated against Ringworm (Rinvag Bovilis®, MSD Animal Health Srl, Milan) and underwent chemoprophylaxis for Leptospirosis (Streptomicina, IZO, Brescia, Italy).

2.2. Study design

At the farms of origin, the bulls were always housed in couples, only five subjects being kept alone in their pens. On entering the ANAFI genetic center, bulls were transferred to a quarantine pen. This included subjects arriving on the same day from other farms, so that the total number ranged from 12 to 14 subjects/pen. Selected immunological parameters were monitored over 30 days after the arrival. In particular, we investigated the time-course of serum bactericidal activity (BA), hemolytic complement (HC), albumin, α, β, and γ-globulins, interleukin (IL)-6 and Tumor Necrosis Factor −alpha (TNF-α). In order to include the effects of both farm and journey, the first blood sample (time,T,−4) was collected four days before transportation to define baseline values, and the second one (T0) immediately after unloading bulls from the truck. Three further samplings took place at days four (T4), fifteen (T15) and thirty (T30) after shipment. Moreover, to evaluate the effects of climate on animals, the shipping and farming procedures were carried out in 3 different periods, i.e., December and January (groups 1 and 2), April (group 3), June and July (groups 4 and 5). Climatic conditions during these periods were defined by calculating the temperature humidity index (THI). The THI incorporates the effects of both temperature and relative humidity into one figure, and it is commonly used to quantify the degree of heat stress on farm animals. Values of maximum and minimum THI were calculated as described before (Vitali et al., 2009), using temperature and relative humidity data recorded at 12 am at a weather station 4 km far from the ANAFI center (Table 2).

2.3. Laboratory analyses

Ten ml of blood/animal were collected from the jugular vein in lithium heparin tubes and in tubes without anticoagulant, respectively. The heparinized blood samples were centrifuged at 1286 × *g* for 15 min and the resulting plasma was stored in aliquots at −20 °C for subsequent analyses. Non-heparinized blood samples were stored at room temperature for 2 h and centrifuged at 1286 × *g* for 15 min; serum aliquots were stored at −80 °C until they were tested. In particular, we measured serum albumin, α, β, and γ-globulins by electrophoresis on cellulose acetate strips and densitometric evaluation of protein bands (Sebia Hydrasis LC, kit Hydrasis Hydragel Protein 15, code PN 4120, Lisses, France). BA and HC (alternative pathway) were determined as previously described (Amadori et al., 1997). Bioassays were used to measure interleukin (IL)-6 (Grenett et al., 1991) and TNF-α (Asai et al., 1993). The cytokine tests were calibrated by means of standard curves created with reference cytokine preparations (R&D system, Minneapolis, USA, Cat. DY8190 e DY2279). In agreement with our previous data on dairy cattle (Amadori et al., 2015), healthy cattle were expected to show <100 and <160 pg/ml of serum TNF-α and IL-6, respectively.

2.4. Statistical analysis

Clinical immunology and chemistry data were analysed using PROC MIXED for repeated measures of SAS (SAS Institute Inc., Campus Drive, USA). The model included the fixed effect of sampling time, season, farm of origin, sampling time × season and sampling time × farm of origin interactions. The threshold of significance was set at $P < 0.05$. Data were transformed according to an assumption of homogeneity of variance and normality of residuals; bactericidal activity values were squared whereas Haemolytic Complement, TNF-α and IL-6 data were transformed into $\log_{10}$ values. All least square means were back-transformed for presentation to their original scale as arithmetic means.

3. Results and discussion

All the animals under study tested negative for antibody to *Brucella* spp., Blue Tongue, Enzootic Bovine Leucosis and Infectious Bovine Rhinotracheitis viruses. Also, they were skin test-negative for bovine tuberculosis. Accordingly, all of them were admitted into the Genetic Center after the quarantine period. During the whole observation period, no animals showed signs of disease. The effect of origin farm, in agreement with our previous paper (Cafazzo et al., 2012), was not significant ($P > 0.05$) for all the parameters under study except BA; for this reason, BA was removed from the model.

Regarding environmental parameters, the first and second period of observation after the arrival (1–31 December and 23 January–22 February) showed similar temperature, humidity and THI values (Table 2). Concerning the third period of observation (20 April–20 May), thermoneutral parameters were observed as expected (Table 2). Regarding the remaining periods (3 June–3 July and 14 July–13 August) notable increases of THI_{max}, THI_{min}, T_{max} and T_{min} were observed, as expected (Table 2). These periods were classified as hot season. Please notice that critical THI values for Friesian cattle (Bernabucci et al., 2015) were overstepped in both summer periods, which may underlie an inflammatory cytokine response (Peli et al., 2013). Yet, the minimum THI data imply that cattle could adequately rest and recover at night. A seasonal effect was observed in the calves arrived at the ANAFI Center for IL-6, TNF-α, albumin and alpha-globulin serum fractions, as opposed to beta and gamma-globulins (See Figs. 1 and 2) .

Albumin, alpha and beta-globulins showed parallel, season-dependent fluctuations, the highest levels being observed in thermoneutral April for alpha-globulins and albumin. Albumin is a negative acute phase protein, and the observed data would be in agreement with minor climate-related changes within the normal physiological values of cattle (Barta, 1993). Alpha-globulins mainly include α1-antitrypsin, α1-antichymotrypsin and α2-