Livestock Science 176 (2015) 152–160

Contents lists available at ScienceDirect

Livestock Science

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

Association of natural (auto-) antibodies in young gilts with osteochondrosis at slaughter

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ARTICLE INFO

Article history:
Received 26 January 2015
Received in revised form
6 March 2015
Accepted 22 March 2015

Keywords:
Gilts
Naturally occurring antibodies
Osteochondrosis

ABSTRACT

Osteochondrosis (OC) develops at a young age and has been associated with lameness and reduced longevity of sows. Early detection of OC is therefore beneficial for selection against OC. Possibly, immunological components within the blood may serve as an indicator for OC development and could therefore be used as a biomarker. Levels of naturally occurring (auto-) antibodies (N[A]Ab) have been associated with homeostatic imbalance and various forms of inflammation, and may have an association with OC. The aim of this study was to investigate possible associations between the presence and levels of N(A)Ab of the IgM and IgG isotypes at an early age with OC in growing gilts at slaughter (24 weeks of age). Plasma samples were obtained from 212 Topigs 20 (Dutch Large White x Dutch Landrace) gilts at 6, 10, and 24 weeks of age and analyzed for N(A)Ab titers against 11 (auto-) antigens using ELISA. After slaughter, the elbow, hock, and knee joints were macroscopically examined for OC status. Due to low prevalence of OC in the elbow joint (5.4%), the elbow joint was not taken into account in analyses. Significant ($P \leq 0.05$) associations with OC in both the hock joint and at the animal level (all joints combined) were found for IgM titers against chondroitin sulfate A at 6 weeks of age (OR 1.4 and 1.5), actin at 6 weeks of age (OR 1.4 and 1.3), thyroglobulin at 24 weeks of age (OR 1.5 and 1.3), and IgG titers against insulin at 6 weeks of age (OR 1.7 and 1.4). Additionally, significant ($P \leq 0.05$) associations with OC were found at the knee joint for IgM titers against albumin at 6 weeks of age (OR 2.3), at the hock joint for IgM titers against keyhole limpet hemocyanin at 6 weeks of age (OR 1.4), and at the animal level for IgM titers against actin at 24 weeks of age (OR 1.3). This study indicated for the first time associations between the presence and levels of N(A)Ab at a young age and OC at 24 weeks of age in breeding gilts.

1. Introduction

Osteochondrosis (OC) has been associated with leg weakness, lameness, and reduced longevity of pigs (Dewey et al., 1993; Jørgensen and Sørensen, 1998; Yazdi et al., 2000). Detecting OC at early age would benefit selection against animals with increased risk for OC, especially breeding and multiplier sows that have to last

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http://dx.doi.org/10.1016/j.livsci.2015.03.017
1871-1413/

several parities. However, early detection methods of OC at young age seems limited.

Osteochondrosis involves the formation of necrotic cartilage due to vascular disruption in epiphyseal growth cartilage which is suggested to occur around 10 weeks of age with a time frame of several weeks (Ytrehus et al., 2004a, 2004b, 2004c; Olstad et al., 2014), and has been associated with the presence of cartilage breakdown products, hormones, and minerals within the blood (Sloet van Oldruitenborgh-Oosterbaan et al., 1999; Billinghurst et al., 2004; Gangl et al., 2007; de Grauw et al., 2011). Natural (auto-) antibodies (N[A]Ab) are defined as being present within the body without prior specific immune activation or antigenic challenge (reviewed by Avrameas, 1991; Avrameas et al., 2007) and have been implicated in maintaining homeostasis of the body by clearing debris from damaged cells (reviewed by Lutz et al., 2009; Binder, 2012; Elkon and Silverman, 2012) thereby preventing or reflecting inflammation. Presence and (changing) levels of N(A)Ab might thus be a possible new serological marker for increased risk of OC in pigs. This could provide evidence for a role of the immune system in the occurrence of OC as suggested for horses and pigs (Osborne et al., 1995; Rangkasenee et al., 2013).

The aim of this study was to assess whether levels of the IgM and IgG isotypes of N(A)Ab are present in young gilts and are associated with OC at slaughter over the previously described and modeled effects of floor type (de Koning et al., 2014). Levels of N(A)Ab might then add to predict risk of OC affliction in breeding gilts at an early age.

2. Materials and methods

2.1. Animals and treatments

This association study is part of a study that assessed the age dependent effects of floor type on OC prevalence (de Koning et al., 2014). The experiment consisted of 212 Topigs 20 (Dutch Large White x Dutch Landrace) gilts acquired from a commercial breeding company (TOPIGS, Veldhuizen Wehl, Wehl, the Netherlands). Gilts were assigned to 1 of 4 treatments and 1 of 32 pens of 6 to 7 individuals after weaning at on average 4 weeks of age, based on an equal distribution of littermates and body weight measured 1 week before the start of the experiment. Gilts from 1 dam were equally divided over treatments as much as possible to prevent that 1 litter received only 1 treatment. Pens consisted of 8.37 m^2 of surface area and were equally divided over 4 departments. Gilts were housed either on a 60% slatted floor and 40% solid floor (conventional) or on 25–50 cm deep bedding of wood shavings. Briefly, treatments consisted of a conventional floor from weaning until slaughter (CC); wood shavings as bedding from weaning until slaughter (WW); a conventional floor from weaning until 10 weeks of age after which gilts were switched to wood shavings as bedding (CW); wood shavings as bedding from weaning until 10 weeks of age after which gilts were switched to a conventional floor (WC). Manure areas were removed as much as possible up to 4 times a week, after which wood shavings were replenished. Gilts were given ad libitum access to water and feed. For further details see de Koning et al. (2014).

2.2. Antibody titer assessment

2.2.1. Blood sampling

Blood samples were collected in EDTA tubes before the treatment switch at 6 and 10 weeks of age from the jugular vein and at slaughter at 24 weeks of age. Blood samples were centrifuged for 10 min at 3000*g* at 4 °C and plasma was stored at −20 °C until use.

2.2.2. Enzyme linked immunosorbent assay

The IgM and IgG antibody titers were assessed by indirect two-step enzyme linked immunosorbent assay (ELISA) against 11 exo- and auto-antigens: keyhole limpet hemocyanin (KLH), bovine actin (ACT), ovalbumin (OVA), and porcine albumin (ALB), -hematin (HEMA), -hemoglobin (HEMO), -thyroglobulin (THYRO), -chondroitin sulfate A (CS-A), -insulin (INS), and –myosin (MYO), all from Sigma-Aldrich Co. LLC. (St. Louis, Missouri), and egg white lysozyme (LYSO) (Merck Millipore, Billerica, Massachusetts), as shown in Table 1.

Ninety-six-wells ELISA microtiter medium binding plates (Greiner Bio-One GmbH, Frickenhausen, Germany) were coated with antigens diluted in carbonate/bicarbonate buffer (0.05 M Na_2CO_3, 0.05 M $NaHCO_3$ pH 9.6) as specified in Table 1. Plates were incubated overnight at 4 °C and subsequently washed with tap water containing 0.05% Tween 20. For assessment of antibody titers against HEMA, HEMO, MYO, and THYRO, an additional blocking step was done with 2% horse serum during 1 h prior to administration of plasma samples. Optimal dilutions for samples and standard controls were determined beforehand. Plasma samples were administered to the plates in 4-step serial dilutions at a 1:3 ratio with a starting dilution of 1:30 in phosphate buffered saline (PBS) pH 7.2 containing 0.5% horse serum and 0.05% Tween 20. In the final assessment of the IgG antibody titers against CS-A, MYO, and INS, the plasma samples were diluted in PBS buffer with bovine serum instead of horse serum and administered to the plates with a 1:4 starting dilution and 4-step serially diluted at a 1:2 ratio. Plasma earlier collected from a sow served as a standard control in two columns on all plates (standard controls). Starting dilutions of the standard controls which were 8-step serially diluted at a 1:2 ratio are displayed in Table 1. After administration of plasma samples and standard controls, plates were incubated at room temperature for 1.5 h and subsequently washed with tap water containing 0.05% Tween 20. Isotype specific peroxidase conjugated anti-porcine antibodies were administered to the plates 1:40,000 diluted for IgG (horse radish peroxidase [PO] conjugated goat anti porcine IgG-Fc, Bethyl Lab. Inc., Montgomery, Texas), and 1:20,000 diluted for IgM (IgM/PO, Bethyl Lab. Inc., Montgomery, Texas) in PBS containing 0.5% horse serum and 0.05% Tween 20, and incubated for 1.5 h. After washing, sodium acetate buffer pH 5.5 with tetramethylbenzidine and urea hydrogen peroxide (comparable with 0.05% H_2O_2) were added and incubated for 10 min at room temperature to initiate the enzymatic color reaction. The reaction was stopped with 50 μL of 1.25 M H_2SO_4. Optical density (OD)