Veterinary Parasitology 198 (2013) 345–350

Contents lists available at ScienceDirect

Veterinary Parasitology

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

Gastrointestinal parasite control during prepuberty improves mammary parenchyma development in Holstein heifers

Adrián F. Perri[a,b], Miguel E. Mejía[a], Nicolás Licoff[a], Santiago S. Diab[c], Néstor Formía[d], Ana Ornstein[a], Damasia Becú-Villalobos[a], Isabel M. Lacau-Mengido[a,*]

[a] Laboratorio de Regulación Hipofisaria, Instituto de Biología y Medicina Experimental, Vuelta de Obligado 2490, 1428 Ciudad Autónoma de Buenos Aires, Argentina
[b] Facultad de Ciencias Veterinarias, Universidad Nacional del Litoral, Esperanza, Santa Fe, Argentina
[c] Animal Health and Food Safety Laboratory, San Bernardino Branch, University of California, Davis, CA, USA
[d] Escuela Inchausti, Universidad Nacional de La Plata, 25 de mayo, Provincia de Buenos Aires, Argentina

ARTICLE INFO

Article history:
Received 29 July 2013
Received in revised form
11 September 2013
Accepted 13 September 2013

Keywords:
Gastrointestinal parasites
Puberty
IGF-1
Mammary development
Dairy heifers

ABSTRACT

Parasitism during development impairs normal growth and delays the onset of puberty through altered hormone profiles, including insulin-like growth factor one (IGF-1). As mammary gland development during prepuberty is strongly dependent on IGF-1, we determined if antiparasitic treatment during this stage of growth improved mammary gland development. One group of Holstein heifers was treated monthly, rotationally with antiparasitic drugs from birth to 70 weeks of age, a second group was untreated. Treated heifer calves had between 56% and 65% less EPG counts than untreated ones. Presence of *Ostertagia*, *Cooperia*, *Haemonchus* and *Trichostrongylus* was demonstrated. Treatment effectively advanced the onset of puberty and increased IGF-1 levels. At 20, 30, 40 and 70 weeks of age biopsies from the mammary gland were taken and histological sections were prepared and stained with hematoxylin–eosin. Pictures were analyzed to compare parenchyma area in relation to total mammary tissue between groups. Mammary samples from treated heifers had higher ratios of parenchyma/total area than untreated ones. As mammary development during prepuberty is crucial for mammary performance during lactation, these results add new evidence to the importance of gastrointestinal parasite control in heifers.

1. Introduction

We have previously demonstrated that ivermectin treatment during early development in grazing dairy heifers, advanced age at puberty and production onset while decreasing culling rate during the first lactation (Mejía et al., 1999, 2009). Furthermore, parasites in cows during the periparturient period were related to impaired milk production (Perri et al., 2011). The effects of gastrointestinal parasites, both on development and production itself, were associated with altered profiles of circulating metabolic hormones (Lacau-Mengido et al., 2000, 2009; Díaz-Torga et al., 2001; Perri et al., 2011). Maximal levels of IGF-1 reached during prepuberty were lower in untreated than in ivermectin treated heifers (Lacau-Mengido et al., 2000) and time of prepubertal increase in serum leptin levels was delayed in untreated heifers (Díaz-Torga et al., 2001). In adult lactating cows, diminished milk production in cows with positive nematode egg counting in feces around parturition was associated with decreased prolactin, GH, and IGF-1 levels (Perri et al., 2011).

* Corresponding author. Tel.: +54 11 4783 2869; fax: +54 11 4786 2564.
E-mail address: ilacau@ibyme.conicet.gov.ar (I.M. Lacau-Mengido).

0304-4017/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.vetpar.2013.09.006

On the other hand, mammary gland development during the peripubertal period has been shown to be dependent not only on ovarian control by estradiol stimulus, but also on the IGF-1 system and its local components (Akers et al., 2005). This growth factor strongly stimulates parenchymal cell division during early mammary development and synergizes with estradiol for ductal growth and branching stimulation (Hinck and Silberstein, 2005). Within the gland there is paracrine interaction between stromal and parenchymal cells *via* growth factors and their receptors which are regulated by extra mammary hormones, such as pituitary, thyroid and ovarian hormones (Forsyth, 1991).

Mammary gland development during the peripubertal period can markedly affect the future milk yield potential (Sejrsen and Purup, 1997). Thus, for scientists seeking to optimize milk production in dairy animals, the peripubertal period has been of increasing interest in recent years. In the present work, considering that parasites lowered IGF-1 during prepuberty and that this hormone is strongly involved in mammary development, we wished to investigate if parasite presence in prepubertal animals can modify mammary development, in addition to their effect on sexual maturity. Because ivermectin resistance has already been demonstrated in Argentina (Anziani et al., 2001), an alternative rotational drug protocol was used for parasite control and the effect on puberty onset and IGF-1 levels was also evaluated.

2. Materials and methods

The experiment was conducted at the dairy farm of the Experimental School of Inchausti, 25 de mayo, Province of Buenos Aires, Argentina (35°36′ S, 60°32′ W). Forty female Holstein calves were randomly assigned, at birth, to an untreated control group (C) or to the treated group (T) which received, from birth onward, monthly anthelminthic treatment rotating different drugs in order to minimize parasite burden, and mitigate drug resistance. The following drugs were used: ivermectin (I, 0.63 mg/kg, Ivomec Gold®, Merial), fenbendazole (F, 7.5 mg/kg, Axilur®, MSD) and levamisole (L, 10 mg/kg, Ripercol L®, Pfizer SRL). The sequence was L, F, F, F, I; F, F, F, L, L, I, L, L, F, F, F, I, starting in August (Winter). The schedule was reasoned as follows: ivermectin was used against inhibited Ostertagia (in December) and a dose in Winter to rotate with levamisole and protect the animals against mange. Levamisole was used in the Winter months when there is no inhibition of Ostertagia. Fenbendazole was used in Spring and Summer to rotate with the other drugs.

All the heifers were raised together with the replacement herd of the dairy farm, outdoors on infected pastures (always assigned to rearing calves) and supplemented with milk and/or concentrate or corn or silage according to age. At birth, calves were placed in individual cages, directly on the pastures, which were moved when the floor turned dirty or humid. They stayed in cages during the first 2 months of life, and during this time they were fed 2 l of warm milk twice a day and had *ad libitum* access to balanced supplement. Then, they were included in the grazing herd and grazed on alfalfa and (or) ryegrass pastures, in a rotational grazing system (stocking density: 8 animals/Ha), with *ad libitum* access to the supplement. At 160 kg of BW the supplement was changed to corn (2 kg/animal day) and the stocking density was reduced to 2 animals/Ha.

At 20, 30, 40 and 70 weeks of age jugular blood and fecal samples were individually obtained from all the heifers in the study, for serum IGF-1 determination by RIA and nematode egg counting in feces. At the same time, mammary biopsies were taken from 6 heifers in each treatment group (Licoff et al., 2009). Heifers were sedated with acepromazine 1% (0.15 mg/kg, Holliday Scott S.A.) and immobilized in the supine position. The udder was cleaned with soap and water, rinsed and disinfected with an iodine solution. The biopsy was taken two centimeters away from the nipple in the rear right quarter under local anesthesia (5 ml lidocaine 2% sc). Each biopsy (at 20, 30, 40 and 70 weeks) was taken in a different place, rotating in clockwise sense from frontal direction, to avoid previous cicatricial tissue. A Tru-Core™ I fully automatic biopsy gun provided with a Tru-Core® 14 G X 20 cm needle (Medical Device Technologies, Inc.) was used. The needle directly pierced the skin, and a gland sample of 3 mm wide per 1 cm long was obtained with minimal lesion.

Additional weekly bleeding was performed in all the heifers between 28 and 48 weeks of age for progesterone and time of puberty onset determination. When progesterone exceeded 1 ng/ml, heifers were considered to be puberal (Mejía et al., 1999).

2.1. Sample treatments

Fecal samples were maintained at 4 °C until examination. Blood samples were centrifuged and sera stored at −20 °C for hormone determination. Mammary gland samples were immediately fixed in buffered formaldehyde 4%, for histological studies.

2.2. Nematode egg counting

Nematode eggs were counted in fresh fecal samples by the Mc. Master method adapted by INTA, with a sensitivity of 10 eggs and expressed as number of eggs per gram of sample (EPG) (Mejía et al., 1999). Cultures were performed in pooled samples, at each biopsial date, to identify the genera of the larvae. Those were *Cooperia*, *Ostertagia*, *Trichostrongylus* and *Haemonchus*.

2.3. Hormone determinations

The IGF-1 RIA was performed after acid–ethanol extraction as described in Lacau-Mengido et al. (2000). The IGF-1 antibody (UB2-495) of the NIDDK was used. Intra-assay coefficient of variation was 8%, and minimum detectable concentration was 2.5 ng/ml. Progesterone was determined with a RIA using antibody provided by G. D. Niswender, after double extraction with hexane. Labeled hormone (progesterone [1,2,6,7 3H(N)]) was purchased from Dupont NEN (Boston, MA). Assay sensitivity was 50 pg, and intra- and interassay coefficients of variation were less than 8% and 12%, respectively.