Veterinary Parasitology 168 (2010) 51–59

Contents lists available at ScienceDirect

Veterinary Parasitology

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

# The periparturient relaxation of immunity in Merino ewes infected with *Trichostrongylus colubriformis*: Endocrine and body compositional responses

A.M. Beasley [a,b,*], L.P. Kahn [b], R.G. Windon [c]

[a] *Australian Sheep Industry CRC, CJ Hawkins Homestead, University of New England, Armidale, NSW 2350, Australia*
[b] *Centre for Animal Health and Welfare, School of Environmental and Rural Science, University of New England, Armidale, NSW 2351, Australia*
[c] *CSIRO Livestock Industries, F.D. McMaster Laboratory, Locked Bag, Armidale, NSW 2350, Australia*

ARTICLE INFO

*Article history:*
Received 22 July 2009
Received in revised form 24 November 2009
Accepted 10 December 2009

*Keywords:*
Periparturient
*Trichostrongylus colubriformis*
Endocrine
Body composition
Leptin
Cortisol
Prolactin
Early weaning

ABSTRACT

The temporal association between the periparturient rise in worm egg count of grazing Merino ewes to infection with *Trichostrongylus colubriformis* and the underlying causal mechanisms was investigated in an experiment that incorporated two levels of pregnancy (pregnant or unmated), two levels of infection (infected with 6000 *T. colubriformis* $L_3$/week or uninfected) and, following lambing, three levels of lactation (non-lactating/"dry", early-weaned 2 days after parturition, or suckled). In addition to parasitological and host immune responses reported in a previous paper (Beasley et al., 2010), a range of endocrine and body compositional changes were monitored from day −50 to day 42, relative to the midpoint of lambing (day 0).

By day −19, pregnant ewes had begun to mobilise fat and eye muscle, and after 42 days of suckling had lost 31% and 8%, respectively, of their existing depots. In comparison to non-pregnant (dry) ewes, the endocrine profiles of late-pregnant ewes were characterised by low levels of cortisol and prolactin and high levels of progesterone and oestradiol. Lactating ewes had lower levels of cortisol and leptin and higher levels of prolactin compared to both dry and early-weaned ewes. The mobilisation of fat and protein reserves throughout lactation in suckled ewes was closely associated with leptin and cortisol profiles, and provided strong evidence of an underlying nutritional basis for the periparturient relaxation of immunity. Both leptin and cortisol concentrations were also associated with both parasite burden and the immune status of the ewe. It is suggested that lower blood cortisol levels in suckled ewes contribute to a Th1 biased immune response that leads to an increased susceptibility to gastrointestinal nematodes. The results provide a detailed characterisation of the physiology underlying the periparturient relaxation of immunity to *T. colubriformis*, from which further investigations will aim to expose potential causal factors.



## 1. Introduction

The underlying physiology which results in the periparturient relaxation of immunity (PPRI) to gastrointestinal nematodes (GIN) in sheep and, ultimately, the periparturient rise (PPR) in faecal worm egg count (WEC) remains poorly understood. As a consequence, the underlying causal factors responsible for the initiation and maintenance of the PPR have yet to be clearly defined. The

* Corresponding author at: Centre for Animal Health and Welfare, School of Environmental and Rural Science, University of New England, Armidale, NSW 2351, Australia. Tel.: +61 7 5466 1340.
*E-mail address:* beasleyab@bigpond.com (A.M. Beasley).

0304-4017/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.vetpar.2009.12.012

approach generally adopted to investigate the PPR has been to focus on WEC or worm burden. However, the physiological changes that underpin initiation of the PPR occur earlier in gestation, before changes in WEC are evident, and predispose the breeding ewe to parasite susceptibility via a relaxation of immunity (Connan, 1968). It is crucial to identify these contributing physiological conditions in order to develop control strategies based on manipulation of host responses.

While the primary causal agents have not been identified, factors that regulate the magnitude of the PPR, such as the supply of metabolisable protein (Donaldson et al., 1997, 1998, 2001; Houdijk et al., 2000; Kahn et al., 2003a,b), host genotype (Woolaston, 1992) and certain hormones such as prolactin (Gibbs, 1967; Bryant et al., 1968) have been described. Although it is a commonly expressed viewpoint that the PPR most likely eventuates from complex interactions between the endocrine and immune systems (Crofton, 1954; Dunsmore, 1965; O'Sullivan and Donald, 1970), the literature provides no documented evidence for this. Furthermore, these interactions may be, in turn, influenced by the nutritional environment and metabolic status of the animal.

The aim of this experiment was to examine the multi-layered physiology underlying the PPR, and identify the factors responsible for its initiation and/or maintenance. We have previously described (Beasley et al., 2010) the parasitological and immunological responses from a large comprehensive data set, and the temporal associations between the PPR and the relaxation of components of systemic and local immunity. This paper extends the results by exploring hormones, body composition and nutrition in modulating the periparturient relaxation of immunity (PPRI). Identification of the contributing mechanisms will allow more targeted future research.

## 2. Materials and methods

### 2.1. Animals, experimental design and infection details

Full details of the animals used, experimental design and infection details have been described in Beasley et al. (2010).

### 2.2. Animal sampling

Individual ewe live weights were recorded on a fortnightly basis from days −51 to −23, then weekly until day 42. Fat score, a subjective measure of soft tissue depth at the "GR" site located 110 mm from the midline over the 12th rib (White and Holst, 2006), was recorded on days −51, −37, −9, 6, 19 and 33. Ewes were scored on a scale of 1 (leanest) to 5 (fattest), using two intermediate points between integer scores (e.g. 3.00, 3.33, 3.66, 4.00). At days −19, 9 and 37, ewes were ultrasound scanned by an industry accredited operator (service provided by Advanced Livestock Services, VIC, Australia) to measure fat depth (FD) and eye muscle depth (EMD) at the "C" site, located 40–45 mm from the midline over the 12th/13th rib (Meat & Livestock Australia, 2005).

Blood was sampled by jugular venipuncture (EDTA vacutainers, Becton Dickinson, Australia) for determination of hormone levels. Sampling occurred fortnightly from days −51 to −23, weekly from day −23 until the start of lambing, 3 times weekly from days −9 to 9, then weekly for the remainder of the experiment. Blood samples were centrifuged at 2000 rpm ($721 \times g$) for 20 min and the plasma portion stored at −20 °C until analysis.

### 2.3. Hormone analysis

The Access® 2 Immunoassay System (Beckman Coulter; CA, USA) was used for the quantitative determination of progesterone, oestradiol and cortisol levels in plasma. The inter-assay coefficient of variation for progesterone, oestradiol and cortisol were 6.4%, 5.7% and 7.7%, respectively.

Measurement of circulating prolactin and leptin concentrations in ewe plasma was conducted at the School of Animal Biology, University of Western Australia. Samples were assayed for prolactin in duplicate by double-antibody homologous RIA as previously described by McNeilly and Andrews (1974). The limit of detection for prolactin in plasma was 1.1 ng/mL and the inter-assay coefficient of variation was <6%. Samples were assayed for leptin using the double-antibody RIA method of Blache (2000). The limit of detection for leptin in plasma was 0.08 ng/mL and the inter-assay coefficient of variation was <5%.

### 2.4. Statistical analysis

Repeated measures analyses and Pearson product-moment correlations applied to endocrine and body compositional data collected from this experiment were carried out as previously described (Beasley et al., 2010).

## 3. Results

It should be noted that, for the sake of clarity, early-weaned and suckled treatment groups are plotted separately prior to lambing in P2, but these groups did not differ significantly.

### 3.1. Blood hormone profiles

#### 3.1.1. Progesterone

Progesterone levels remained unaffected by infection status during the experiment. At all times during period 1 (P1), pregnant ewes had significantly higher blood concentrations than their dry counterparts ($P < 0.001$) (Fig. 1A). Progesterone levels in pregnant ewes declined rapidly between days −9 and 0. Early weaning of lambs had no effect on blood progesterone concentrations and throughout period 3 (P3), there was no difference between treatment groups.

#### 3.1.2. Oestradiol

Oestradiol levels were unaffected by infection status. Pregnancy status significantly affected blood oestradiol concentration during P1 ($P < 0.001$) with pregnant ewes having higher levels than dry ewes (Fig. 1B). Oestradiol