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Short communication

Pregnancy toxaemia in ewes: Development of an experimental model and potential interactions with gastrointestinal nematode infections

M.S. Barbagianni[a], E. Giannenas[b], E. Papadopoulos[b,*], I.G. Petridis[a], S.A. Spanos[a], P.G. Gouletsou[a], I. Valasi[a], G.C. Fthenakis[a]

[a] Veterinary Faculty, University of Thessaly, 43100 Karditsa, Greece
[b] Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece

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ABSTRACT

Objective of the present study was to develop an experimental model that could be used for the study of pregnancy toxaemia in ewes. The study included 28 ewes, which initially had received effective anthelmintic treatment and, then, received a mixture of trichostrongylid infective larvae. Until the 60th day of pregnancy, per ewe daily ration was 1.30 kg of a concentrate feed (net energy: 0.844 FU_L) plus 2.50 kg of alfalfa hay. From the 60th to 100th day of pregnancy, per ewe daily ration was 0.60 kg of the same concentrate plus 2.00 kg of hay. From the 100th day of pregnancy, per ewe daily ration was 0.50 kg of a reduced energy concentrate feed (net energy: 0.748 FU_L) plus 0.50 kg of alfalfa hay for ewes with one foetus; during that period, respective figures for ewes with two foetuses were 0.60 kg and 0.50 kg and for ewes with three foetuses were 0.80 kg and 0.50 kg. In total, 16 ewes developed increased β-hydroxybutyrate blood concentrations, characteristic of pregnancy toxaemia. There was a significant reverse correlation ($P = 0.016$) between blood β-hydroxybutyrate concentrations and lamb birth bodyweight. Ewes with pregnancy toxaemia had greater faecal epg counts than ewes with no pregnancy toxaemia ($P < 0.025$); there was also a significant reverse correlation between faecal epg counts and lamb birth bodyweight ($P = 0.03$) and a significant correlation between blood β-hydroxybutyrate concentrations and faecal epg counts ($P < 0.001$). Ewes with pregnancy toxaemia also had smaller blood concentrations of glucose than ewes with no pregnancy toxaemia ($P = 0.033$). In conclusion, administration of a concentrate feed with reduced energy content during the last stage of pregnancy induced pregnancy toxaemia in ewes, at the same time covering satiation requirements of the animals and thus maintaining welfare standards. Parasitism might have further contributed to improving the efficacy of the model.

1. Introduction

Pregnancy toxaemia is the most important metabolic disease of pregnant ewes, caused by abnormal metabolism of carbohydrates and fats during the final stage of pregnancy (Mavrogianni and Brozos, 2008; Brozos et al., 2011). Decreased availability of nutrients, coupled with increased energy requirements of the animals during the final stage of gestation, can lead to development of the disorder. The main ketone body found in the blood of pregnant ewes is β-hydroxyburate; its concentration can be used for definitive diagnosis of the disease, as well as to detect animals at risk to develop pregnancy toxaemia. β-hydroxybutyrate concentration is measured in the blood of animals, with the threshold value used to identify animals at risk to develop the disease being 1.1 mmol L^{-1} (Sargison, 1995, 2007; Braun et al., 2010; Brozos et al., 2011). As pregnancy toxaemia is important in sheep flocks worldwide, there is a need for an experimental model to study the disorder. Objective of the present study was to develop a valid model for studying the disease, whilst maintaining appropriate welfare standards in the experimental animals.

2. Materials and methods

2.1. Experimental overview

In total, 28 3–5-year old Chios-cross ewes were included in the study. Conditions prescribed by legislation of the European Union in relation to animal experimentation procedures (Council Directive 86/809/EEC) were met during this work. Throughout the study, ewes were housed. Their feeding regime varied according to stage

* Corresponding author. Fax: +30 2310 999947.
E-mail address: eliaspap@vet.auth.gr (E. Papadopoulos).

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of pregnancy and the number of foetuses borne, as detailed herebelow.

All animals into the study were initially drenched with a broad-spectrum anthelmintic, specifically netobimin(HAPADEX or. dr., Merck Animal Health, Summit, USA; dose rate: 20 mg kg^{-1} bodyweight) 30 days before start of the mating period. Then, 21 days later, the ewes received orally 2 mL of phosphate–buffer–saline containing 5000 third-stage larvae of a trichostrongylid helminth mixture from local strains of *Teladorsagia* spp., *Trichostrongylus* spp., *Cooperia* spp., *Haemonchus* spp. and *Oesophagostomum* spp.

Four rams of known fertility were penned in a box adjoining to the one with the ewes for one month before introduction into the females. They were introduced into the flock of ewes in early August, at which time sheep were in the reproductive season. Animals were observed daily and matings were recorded; all ewes were mated within 20 days after ram introduction and no repeat matings were observed.

Ewes were subsequently monitored throughout their pregnancy. Repeated ultrasonographic examinations were carried out to confirm pregnancy and number of foetuses borne. Blood samples were collected from all ewes at frequent time-points after 100th day of pregnancy for measurement of β-hydroxybutyrate and glucose concentrations. Faecal samples were collected for parasitological examinations. Ewes with clinical signs characteristic of pregnancy toxaemia were recognised for treatment. All ewes lambed 146–169 days after introduction of rams. Finally, on the day of birth, each lamb was weighed.

2.2. Animal feeding

Starting 40 days before ram introduction and until the 60th day of pregnancy, the ration provided per ewe was 0.65 kg of a commercial concentrate feed in mash form plus 1.25 kg of alfalfa hay, offered twice daily. Barley straw was provided into the pens thrice daily, whilst water was available *ad libitum*. The concentrate feed was based on cereal grains, cereal bran and oil by-products; details are in Table 1. From the 60th to the 100th day of pregnancy, the ration provided per ewe was 0.30 kg of the same concentrate plus 1.00 kg of alfalfa hay, offered twice daily. Barley straw and water were provided as above.

On the 100th day of pregnancy, animals were allocated into separate pens according to the number of foetuses borne (one, two or three). In ewes with one foetus, the ration provided per ewe was 0.25 kg of a specially formulated and prepared concentrate feed in mash form plus 0.25 kg of alfalfa hay, offered twice daily. Respective figures for ewes with two foetuses were 0.30 kg and 0.25 kg and for ewes with three foetuses were 0.40 kg and 0.25 kg. Barley straw was provided into the pens thrice daily, whilst water was available *ad libitum*. The concentrate feed was based on cereal grains, cereal bran and oil by-products; details are in Table 1.

Feed was provided in troughs with a space of 40 cm per ewe. Transition from the standard concentrate feed to the specially formulated feed was carried out as follows: for 3 days a mixture of 3:1 standard feed:special feed was given to ewes, for another 3 days a mixture of 1:1 standard feed:special feed was given and, finally, for another 3 days a mixture of 1:3 standard feed:special feed was given.

2.3. Parasitological examination

Faecal samples were initially collected on the day of challenge, 21 days later (9 days before ram introduction into the flock of ewes) and 28 days after ram introduction into the ewes. Then, samples were again collected on the 100th, the 120th and the 140th day of pregnancy. Faecal samples were collected directly from the rectum of each animal, placed into an isothermic box and transferred to the laboratory for epg counting. Each sample was divided in three lots, as follows. One lot was processed for trichostrongylid epg counting according to the modified McMaster technique with saturated NaCl solution; the second lot was processed for *Dicrocoelium dendriticum* epg counting according to the modified McMaster technique with $ZnSO_4$ (sp.g. 1.40); finally, the third lot was processed for *Fasciola* spp. and *Paramphistomum cervi* epg counting by using the Telemann sedimentation technique (acid-ether) (Ministry of Agriculture, Fisheries and Food, 1986; Rehbein et al., 1999; Otranto and Traversa, 2002; Taylor, 2010).

2.4. Blood biochemical tests

Starting on to the 100th day of pregnancy and every 5 days thereafter, a blood sample was collected from each ewe in the study, for measurement of β-hydroxybutyrate and glucose concentrations. On all occasions, samples were collected 4–5 h after the morning feeding of the animals. A drop of blood was placed on an appropriate strip, which was subsequently inserted into an automated reader (Precision Xceed Meter; Abbott Laboratories, Abbott Park, IL, USA), validated for measurement of β-hydroxybutyrate or glucose concentration in sheep blood (Panousis et al., 2012; Pichler et al., 2014). Different strips were used for measurements of β-hydroxybutyrate and glucose concentrations.

2.5. Data management and analysis

Conception day for ewes was considered to be the day of their mating. For statistical analysis, animals into the study were initially grouped according to number of foetuses borne (one, two or three). Retrospectively and based on results of blood β-hydroxybutyrate concentrations, animals were allocated into one of two groups: group A included ewes with β-hydroxybutyrate concentrations $\geq$1.2 mmol L^{-1} in two samples collected after the 129th day of pregnancy, whilst group B included all other ewes into the study. All data were entered into Excel spreadsheets (Microsoft Corporation; Redmond, WA, USA). Initially, descriptive statistics for all parametres and all groups were performed.

Birth bodyweights of lambs born from ewes into the study were compared between groups by using Student's *t*-test.

Repeated measures mixed effect linear regression models were used to determine whether outcomes changed over the course of the study period. Models were adjusted for repeated measures within animals. Independent variables (fixed effects) included (i) number of foetuses borne (one, two or three) or inclusion in group A or B (based on results of blood β-hydroxybutyrate concentrations) and (ii) day of pregnancy, as well as a day of pregnancy by number of foetuses borne or group A/B.

Analysis of correlation between blood β-hydroxybutyrate concentrations and faecal epg counts / blood glucose concentrations / lamb birth bodyweight and between faecal epg counts and lamb birth bodyweight was performed.

All analyses were performed by a commercial statistical program (SPSS, v. 15 for Windows; SPSS Inc., Chicago, IL, USA). Significance level was set at $P = 0.05$.

3. Results

3.1. Clinical findings

All ewes into the study conceived; 7 of them bore one foetus, 17 bore two foetuses and 4 bore three foetuses. In 4 ewes (1 with two foetuses and 3 with three foetuses) (14% of ewes into the study), clinical signs characteristic of pregnancy toxaemia were recorded after the 140th day of pregnancy. These included