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Trematode infections in pregnant ewes can predispose to mastitis during the subsequent lactation period

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ABSTRACT

Objective was to investigate if trematode infections predispose ewes to mastitis and/or metritis. We used 80 trematode-infected ewes: primigravidae in group P-A and multigravidae in M-A remained untreated, primigravidae in P-B and multigravidae in M-B were drenched with netobimin and multigravidae in M-C were given rafoxanide. We collected faecal samples for parasitological examination, blood samples for β-hydroxybutyrate concentration measurement and uterine content, teat duct material and milk samples for bacteriological examination. We found significant differences in blood β-hydroxybutyrate concentrations between M-A, M-B and M-C during pregnancy ($P \leqslant 0.002$). We did not observe significant differences between groups regarding development of metritis ($P > 0.83$). We found that for M-A, M-B and M-C ewes, respectively, median time to first case of mastitis was 5.75, 21 and 6.75 days after lambing ($P = 0.003$) and incidence risk of mastitis was 0.308, 0.069 and 0.222 ($P = 0.047$). We postulate that trematode infections predispose ewes to mastitis; perhaps, increased β-hydroxybutyrate blood concentrations adversely affect mammary cellular defences. This is the first report associating parasitic infections with mastitis in sheep.

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1. Introduction

Mastitis and parasitic infections are two significant health problems of sheep. Control programs are available for both conditions, but, nevertheless, the disorders are prevalent in sheep flocks around the world.

Mastitis control programs give emphasis in reducing the rate of mammary infections during milking routine, when mammary glands are at the greatest risk for infection. However, various other factors outside the milking routine (environmental, nutritional, genetic, anatomic) have been identified to predispose ewes to mastitis (Bergonier and Berthelot, 2003; Bergonier et al., 2003; Berthelot et al., 2006). Various diseases can also predispose ewes to mastitis; for example, orf virus infection has been found to cause depletion of cellular defences within the teat duct, hence leading to easier multiplication of invading pathogens and clinical mastitis (Mavrogianni et al., 2006b).

Metritis, another *post-partum* disease of ewes, occurs sporadically (Mavrogianni and Brozos, 2008), often as the consequence of dystocia or retention of foetal membranes (Tzora et al., 2002). Due to the sporadic nature of the disease, there are no routine control programs for its prevention, although strategic administration of antibiotics to ewes immediately after lambing has been found to be beneficial (Mavrogianni et al., 2007a).

To the best of our knowledge, possible associations between parasitic and *post-partum* infections in ewes have not been reported thusfar. Objective of the present work was to study the possible role of trematodes, which commonly cause parasitic infections, in predisposing ewes to metritis or mastitis *post-partum*.

2. Materials and methods

2.1. Study design and animals

The study was performed in a dairy flock in Central Greece. At the beginning of the autumn (D-134) prior to the main study, 152 Lacaune-breed, female sheep in the flock were exposed to the 'ram effect', in order to achieve some degree of synchronisation of oestrous cycles. Then, rams of confirmed fertility were introduced into the females (ram:ewe ratio = 1:15) on the 1st October (D-104), for lambings to take place in the subsequent spring.

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Animals for inclusion in the study were chosen at the end of November (D-46), among those that had been mated, but had not returned to oestrus. At the time of selection, the udders of all animals were clinically examined (Fthenakis, 1994; Saratsis et al., 1998); animals with mammary abnormalities were excluded from the study. At the end, 30 primigravidae (P) and 50 multigravidae (M) ewes were selected for inclusion in the study and, on the same day, were drenched with moxidectin (CYDECTIN, Pfizer Animal Health; dose rate: 0.2 mg kg^{-1} bodyweight).

On the 13th January (D 0), i.e., at around 3–3.5 months of pregnancy, the 80 animals were drenched again with moxidectin. Then, they were allocated into five groups and treated as follows; primigravidae ewes in group P-A ($n = 15$) remained untreated; primigravidae ewes in group P-B ($n = 15$) were drenched with netobimin (HAPADEX or. dr., Intervet–Schering-Plough, Boxmeer, The Netherlands; dose rate: 20 mg kg^{-1} bodyweight); multigravidae ewes in group M-A ($n = 15$) remained untreated; multigravidae ewes in group M-B ($n = 15$) were drenched with netobimin (HAPADEX or. dr.; dose rate: 20 mg kg^{-1} bodyweight); multigravidae ewes in group M-C ($n = 20$) were given rafoxanide (RAFOXANIDE tabs, Provet Animal Health, Alimos, Greece; dose rate: 7.5 mg kg^{-1} bodyweight).

Animals were maintained in a semi-intensive system, grazing in private paddocks during the day and kept indoors during the night. They were also given commercial compound feed, 0.7 kg per animal daily, increased to 1.0 kg per animal daily subsequently to D 20 (i.e., at around 3.5–4 months of pregnancy). Good quality hay was also provided *ad libitum*.

Subsequently to lambing (L 0), uterine content, teat duct material and milk samples were collected on two, three and three occasions, respectively, for bacteriological examination.

As part of planned health program, dogs in the flock had been given anthelmintics (including praziquantel) at regular intervals for the last three years before start of the study. Moreover, monitoring of routinely-slaughtered animals had not, for many months, revealed evidence of parasitic cystic formations in the liver of the flock's sheep.

2.2. Samplings and examinations

2.2.1. Samplings for parasitological monitoring

Faecal samples from all 152 females in the initial batch were first collected on D-134, in September before start of the mating period; samples were again collected on D-63, in mid-November. Thereafter, samples were collected only from the 80 animals into the study, on D-31 (mid-December), on D 0 (mid-January), on D 28 (mid-February), immediately *post-partum* (L 0–L 1, D 41–D 57) and on D 97 (L 40–L 57).

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 epg counting of *Fasciola* spp. and helminthes of the Paramphistomatidae family 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).

2.2.2. Samplings for measurement of β-hudroxybutyrate concentration

On D 0 (mid-January), on D 28 (mid-February), immediately *post-partum* (L 0–L 1, D 41–D 57) and 35–40 days *post-partum* (D 75–D 97), a blood sample was collected from each ewe in the study, for measurement of β-hydroxybutyrate concentration. In all the occasions, samples were collected 6–7 h after the morning feeding of the animals. A drop of blood was placed on a strip, which was subsequently inserted into an automated reader (Precision Xceed Meter; Abbott Laboratories, Abbott Park, IL, USA), validated for measurement of β-hydroxybutyrate concentration in sheep blood (Panousis et al., 2012).

2.2.3. Samplings for monitoring uterine or mammary infection

Immediately *post-partum* (L 0–L 1, D 41–D 57), a general clinical examination of each ewe was carried out (Lovatt, 2010). Special attention was given to the genital system and the mammary glands. A sample of uterine content was collected from each ewe, by placing a sterile catheter into the uterus and sampling with a sterile swab. Teat duct material samples were collected from each teat, by using a fine (20 G), plastic, sterile catheter (Abbocath; Abbott, Abbott Park, IL, USA) according to the method described in detail by Mavrogianni et al. (2006a). Milk samples were collected from each mammary gland into sterile containers. Sampling procedures were as described before (uterus: Tzora et al., 2002; Mavrogianni et al., 2007a – teat ducts and mammary glands: Fthenakis, 1994; Mavrogianni et al., 2005).

The genital tract was re-examined and sampled on L 2–L 3; the teats and mammary glands were re-examined and sampled on L 13–L 15 and on L 35–L 40. In between samplings, animals were monitored and, if a case of clinical metritis or mastitis developed, affected animal was individually examined and appropriately sampled as detailed above.

Uterine content, teat duct material and milk samples were cultured onto Columbia sheep blood agar. The media were incubated aerobically, anaerobically and in a CO_2 environment for 24 h; if nothing had grown, they were reincubated for another 48 h. Bacterial identifications were performed by using standards methods (Barrow and Feltham, 1993; Euzeby, 1997). Cytological examination of uterine content samples was carried out by the Diff-Quick (modified Wright) staining method (Saboorian et al., 1997) for estimation of number of leucocytes and proportion of their subpopulations. Cytological examination of milk samples was carried out by the Microscopic cell counting method (IDF reference method) (International Dairy Federation, 1984; Raynal-Ljutovac et al., 2007), the California mastitis test (Fthenakis, 1995) and the Giemsa method, for cell counting and estimation of proportion of leucocyte subpopulations.

2.3. Data management and analysis

The arithmetic mean of epg counts (AM) was calculated as $AM = (\text{count}_1 + \text{count}_2 + \ldots + \text{count}_n)/n$, where n is the number of animals in the group. Results for samplings performed on D-134 and D-63 took into account epg counts only the 80 animals into the study. Analysis of covariance for post-treatment results, using pre-treatment counts as covariate and treatment as fixed effect, was performed in order to compare differences in epg counts between the two groups of primigravidae/primiparous ewes and the three groups of multigravidae/multiparous ewes. The method was applied for *Dicrocoelium* and *Fasciola* epg counts separately.

Analysis of covariance was employed for comparison of results of blood β-hydroxybutyrate concentration, using value on D 0 as covariate and treatment as fixed effect.

In the present study, there is a difficulty with attempts at estimating incidence rate (new 'infection' per individual at risk for each time point at risk). In many cases, a 'site' (uterus, teat duct, or mammary gland) might change from being 'infected' to being 'uninfected' and *vice versa*; therefore, it was not possible to know what happened between the two sampling occasions, i.e., how many infections and 'cures' there might have been. Therefore, the

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