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Research paper

Field evidence for association between increased gastrointestinal nematode burden and subclinical mastitis in dairy sheep

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ABSTRACT

The objective of the present study was to study, under field conditions, potential associations between gastrointestinal nematode parasitism and subclinical mastitis in ewes during the lactation period. Faecal and milk samples were collected from 240 ewes in 16 farms, for parasitological and bacteriological plus cytological examination, respectively. In the population sampled, prevalence of gastrointestinal nematode infection was 63.0%; mean faecal count was 357.7 ± 32.4 eggs per gram (epg); *Teladorsagia* spp. larvae were identified more frequently in coprocultures (median proportion among farms: 53.0%). The prevalence of subclinical mastitis was 22.6%; coagulase-negative *Staphylococcus* spp. were identified more frequently as causal agents (64.7% of isolates recovered from cases of the disease). There was clear evidence that the prevalence of subclinical mastitis was higher among ewes with gastrointestinal nematode infection than among ewes without: 26.4% and 16.1% ($P = 0.047$), respectively. Further, the prevalence of subclinical mastitis was higher in ewes with high faecal epg: 31.1% versus 18.6% in ewes with low faecal epg ($P = 0.027$). Mean epg counts in ewes with mastitis were significantly higher: 500 ± 84 (mean ± standard error of the mean), than epg counts in healthy ewes: 316 ± 36 ($P = 0.024$). The findings further underline the importance of concurrent parasitic and bacterial infections. Moreover, results also suggest further factors that can play a role in development of mastitis in ewes.

1. Introduction

In a previous paper (Mavrogianni et al., 2017), we have described that, in ewes, gastrointestinal nematode parasitism, and particularly *Teladorsagia* spp. infection, might predispose to clinical mastitis. The study described in that paper had been performed using *Mannheimia haemolytica* bacteria for induction of experimental mastitis. In that study, it had been postulated that severe gastrointestinal nematode parasitism would primarily affect cellular defences of animals, thus contributing to development of clinical disease after challenge with the pathogen (Mavrogianni et al., 2017).

The situation in the field might be different. Many other bacteria apart from *M. haemolytica* have been identified as aetiological agents of mastitis in ewes, whilst various predisposing factors increase risk for development of the disease (Gelasakis et al., 2015). The objective of the present study was to evaluate, under field conditions, potential associations between gastrointestinal nematode parasitism and subclinical mastitis in ewes throughout the lactation period.

2. Materials and methods

2.1. Sheep farms

In total, 16 sheep farms in southern Greece were included into the study and visited for collection of samples. Farms were included into the study on a convenience basis (willingness of farmers to accept a visit by University personnel for sample collection), among ones managed under the semi-intensive system (European Food Safety Authority, 2014) with broad-spectrum anthelmintic treatment performed at least 50 to 70 days prior to the visit. In the farms, there were 3465 ewes and 155 rams; ewe population per farm varied from 90 to 370 (mean: 217 ± 20.5) animals. The principal investigators (NGK and GCF) accompanied by an assisting investigator (NGCV or VSM) visited the farms for sample collection.

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2.2. Animal sampling

In each farm, 15 clinically healthy ewes (*secundiparae* or older) were selected at random. For selection of animals, farmers had been asked to remove from the main flock *primiparae* ewes and ewes with known udder abnormalities. Then, 15 ewes were selected for sampling, by using an electronic random number generator (www.randomresult.com), among the first 50 animals as they walked into the milking area (Vasileiou et al., 2018a).

A standardised clinical examination of the udder (observation, palpation, comparison between glands) was performed (Fthenakis, 1994; Mavrogianni et al., 2005) and the first two squirts of secretion were drawn on the gloved hand of an assisting investigator and assessed. All investigators involved in sampling procedures wore disposable, non-sterile latex gloves, which were changed after procedures in each animal had been completed and before moving to the next one. If udder abnormalities (e.g., abnormal secretion, mammary nodules [i.e., firm space-occupying structures], papilloma-type lesions) were to be detected, the ewe were to be excluded from sampling.

The orifice, edge and lower half of the body of the teat were disinfected by single-use sterile gauzes, onto which povidone iodine 7.5% (Betadine surgical scrub®; Mundipharma Medical Company, Basel, Switzerland) had been poured, followed by wiping off by means of a new sterile gauze; different gauzes were used for each teat. Then, 10 to 15 mL of secretion were collected into a sterile container; separate samples were collected from each mammary gland into separate containers. Milk samples were then drawn directly onto a paddle for performing the California Mastitis Test (CMT).

Finally, faecal samples were collected directly from the rectum of each animal. At least 50 g of faeces were collected in each sample.

All samples were stored into portable isothermic boxes with ice packs and transported by car.

2.3. Microbiological examination

Laboratory procedures started within 12 h after collection. Milk samples (10 μL) were cultured using Columbia blood agar plates incubated aerobically at 37 °C for 48 h. If nothing had grown, media were re-incubated for another 24 h. Bacterial identifications were performed by using standards methods (Barrow and Feltham, 1993; Euzeby, 1997; Vasileiou et al., 2018a).

2.4. Cytological examination

After sample collection, at ewe-side, all samples were tested by use of the CMT. The test was performed as previously described for ewes' milk (Fthenakis, 1995); it was carried out and scored always by the same person, i.e., the principal investigator (NGK). Five degrees of reaction ('negative', 'trace', 'l', '2', '3') were described (Schalm et al., 1971). Milk smears were also produced and dried.

Subsequently, the Microscopic cell counting method (Mccm) (International Dairy Federation reference method) (International Dairy Federation, 1984) was performed in 112 samples (46.7% of all samples). The milk smears were stained by the Giemsa method for estimation of leucocyte subpopulations; proportion of leucocyte types therein was calculated by observing at least 10 fields of each milk film under magnification 10×.

2.5. Parasitological examination

Each faecal sample was divided in three lots, as follows. One lot was processed for trichostrongylid faecal worm egg counts using the modified McMaster technique with saturated NaCl solution (sp.g. 1.20) (minimum detection limit: 50 epg) (Ministry of Agriculture, Fisheries and Food, 1986; Taylor, 2010); the second lot was processed for *Dicrocoelium dendriticum* faecal worm egg counting according to the modified McMaster technique with $ZnSO_4$ (sp.g. 1.40) (Rehbein et al., 1999; Otranto and Traversa, 2002); the third lot was processed for faecal worm egg counting of *Fasciola* spp. and helminths of the Paramphistomatidae family by using the Telemann sedimentation technique (acid - ether) (Foreyt, 2001). Finally, a pooled faecal sample from animals sampled in each farm was cultured and 3rd stage larvae were collected, using the Baermann method (Ministry of Agriculture, Fisheries and Food, 1986), and genus identification was performed always by the same investigators (AK, EP).

2.6. Data management and analysis

Gastrointestinal nematode infection was considered in ewes in which at least one parasitic species / group under investigation was detected in faecal samples. The arithmetic mean of epg (eggs per gram) counts in each farm and in the total population sampled was calculated. For all epg counts, $\log_{10}$ transformations were performed ($\log_{10}[\text{count}_n + 1]$), for use in statistical computations of parasitological data.

Subclinical mastitis was considered in ewes in which a bacteriologically positive milk sample ([a] > 10 colonies of the same organism and [b] no more than two different types of colonies) with concurrently increased CMT score (≥ 'l') and neutrophil and lymphocyte proportion (≥ 65% of all leucocytes) was detected (Fragkou et al., 2014; Vasileiou et al., 2018a). Mammary carriage was considered in ewes in which a bacteriologically positive milk sample with no increased CMT score (≤ 'trace') or neutrophil and lymphocyte proportion (< 65% of all leucocytes) was detected. Isolation of ≤ 10 colonies of an organism or isolation of over two different types of colonies from a sample was considered to indicate contamination (Vasileiou et al., 2018a). The term mammary 'carriage' (or 'carrier state') (Verhoeven et al., 2014) is used to describe presence of bacteria in the mammary gland (identified by isolation in milk samples) with no increased somatic cell numbers (i.e., in the absence of inflammation).

Quantitative information on the cellular content of ewes' milk was obtained by using two sets of data: the CMT results and the results of the Mccm. Although it is generally established that CMT results are reliable proxy measurements for somatic cell counts (SCCs) (Fthenakis, 1995; Gonzalez-Rodríguez and Carmenes, 1996), we further confirmed that in the present study. In analysis of correlation, following assignment of numerical values to CMT scores (value 0 to score 'negative', value 1 to score 'trace', value 2 to score '1', value 3 to score '2', and value 4 to score '3') and $\log_{10}$-transformations, correlation coefficient between CMT scores and Mccm SCCs was $r = 0.942$ (95% Confidence Intervals [C.I.]: 0.917 - 0.959) ($P < 0.001$) and the corrected R^2 was 88.8% (Vasileiou et al., 2018a).

Data were entered into Microsoft Excel for analysis. Basic descriptive analysis was performed. The outcomes of 'gastrointestinal nematode infection' and 'subclinical mastitis' / 'mammary carriage' were considered. Exact binomial confidence intervals were obtained.

The presence of subclinical mastitis (a) in ewes parasitised or not by gastrointestinal nematodes and (b) in ewes with epg counts above or below mean epg count in the population sampled was evaluated in a table of cross-categorised frequency data by use of Pearson chi-square test. Further analyses were performed to evaluate presence of subclinical mastitis in ewes with epg counts above or below (a) 300.0 epg, (b) 600.0 epg and (c) 854.4 epg in the population sampled; the first value had been considered in an earlier study as the threshold for performing targeted anthelmintic treatments in dairy sheep in Greece (Gallidis et al., 2009), the second value was double the previous value and the third value was the mean value in the population sampled plus one standard deviation.

Prevalence of subclinical mastitis in farms with high average epg counts (i.e., > median epg counts in farms into the study) was compared to that in farms with low average faecal epg (i.e., < median epg counts in farms into the study). Further, prevalence of subclinical

mastitis in farms with high proportion of *Teladorsagia* spp. or *Haemonchus* spp. in coprocultures from faecal samples (i.e., > median proportion in farms into the study) was compared to that in farms with low proportion of *Teladorsagia* spp. or *Haemonchus* spp. in samples (i.e., < median proportion in farms into the study). Among ewes with subclinical mastitis, proportion of ewes with bilateral mastitis (i.e., subclinical mastitis, as defined hereabove, in both mammary glands of the sams ewe) in ewes with high or low epg counts (as defined hereabove) was compared by use of Fisher-exact test. The analysis of presence of subclinical mastitis (a) in ewes parasitised or not by gastrointestinal nematodes and (b) in ewes with epg counts above or below mean epg count in population sampled was repeated on individual farm basis by use of Fisher-exact test.

Mean faecal epg counts in ewes with subclinical mastitis and healthy ewes were compared by means of Student's *t*-test. Mean faecal epg counts in ewes with subclinical mastitis caused by coagulase-negative staphylococci were also compared by Student's *t*-test to those in ewes with subclinical mastitis caused by *Staphylococcus aureus* or *M. haemolytica*. Student's *t*-test was also used to compare mean number of bacterial isolates recovered from cases of subclinical mastitis from ewes with high or low (as defined above) faecal epg counts.

All above analyses were repeated for mammary carriage. For all above analyses, ewes in which trematode infection had been detected, were excluded. In all cases, level of significance was set at $P = 0.05$.

3. Results

In total, 240 ewes were examined during the study. All were found to be clinically healthy when sampled.

3.1. Prevalence and aetiology of subclinical mastitis

In total, 53 ewes were detected with subclinical mastitis; its prevalence in the population sampled was 22.6% (95% C.I.: 17.7%–28.3%). Within the study farms, prevalence varied from 6.7% to 53.3% (median value among farms: 20.0%) (Table 1).

In total, 68 bacterial isolates were obtained from ewes with subclinical mastitis, i.e., 1.28 isolates per ewe. Bacteria isolates from ewes with subclinical mastitis were identified as coagulase-negative *Staphylococcus* spp. (n = 44), *S. aureus* (n = 21) or *M. haemolytica* (n = 3).

In 24 ewes, mammary carriage was detected; its prevalence in the population sampled was 10.2% (95% C.I.: 6.9%–14.7%). Within farms in the study, its prevalence varied from 0.0% to 26.7% (median value among farms: 13.3%) (Table 1). In total, 28 bacterial isolates were obtained from ewes with mammary carriage, i.e., 0.12 isolates per ewe. Bacteria isolates from ewes with mammary carriage were identified as coagulase-negative *Staphylococcus* spp. (n = 24), *Streptococcus* spp. (n = 2) or *Corynebacterium* spp. (n = 2).

Table 2
Results of prevalence of infection by gastrointestinal nematodes of sheep population sampled in 16 farms in Greece.

Genus	Prevalence (95% CI) of respective infections among population sampled	Prevalence of respective infections within farms (min-max)
Trichostrongylidae[a]	0.600 (0.537 – 0.660)	0.200 - 0.867
Nematodirus	0.038 (0.020 - 0.070)	0.000 - 0.200
Trichuris	0.038 (0.020 - 0.070)	0.000 - 0.133
Strongyloides	0.029 (0.014 – 0.059)	0.000 - 0.200

[a] Larvae developed and identified in coprocultures: *Teladorsagia* spp., *Haemonchus* spp., *Trichostrongylus* spp., *Cooperia* spp., *Bunostomum* spp., *Chabertia* spp.

3.2. Prevalence of gastrointestinal nematode infection

In total, 148 ewes were found to be infected with gastrointestinal nematodes; prevalence of gastrointestinal nematode infection in the population sampled was 63.0% (95% C.I.: 56.6%–68.9%). Within the study farms, prevalence varied from 20.0% to 85.7% (median value among farms: 70.3%) (Table 1). Details of prevalence of infection by the various parasites are in Table 2.

Mean faecal count in population sampled was 357.7 ± 32.4 epg. Mean faecal counts within farms varied from 20.0 to 823.3 epg (median value among farms: 400.3 epg). Median proportions of parasitic species larvae developed and identified in coprocultures were as follows: *Teladorsagia* spp. 53.0%, *Haemonchus* spp. 38.0%, *Trichostrongylus* spp. 5.0%, *Nematodirus* spp.3.5%, *Bunostomum* spp. 2.5%, *Cooperia* spp. 2.0%, and *Chabertia* spp. 1.0%.

D. dendriticum eggs were detected in samples from 5 ewes. Eggs of *Fasciola* spp. or helminths of the Paramphistomatidae family were not detected in any sample.

Table 1
Detailed results of prevalence of mammary infection and of parasitic infection in 16 sheep farms in Greece.

Farm	Prevalence of				Proportion of parasitic genera found in coprocultures						
	subclinical mastitis	mammary carriage	gastrointestinal nematode parasitism	epg (mean ± s.e.[a])	*Teladorsagia*	*Haemonchus*	*Trichostrongylus*	*Cooperia*	*Chabertia*	*Bunostomum*	*Nematodirus*
1	20.0%	20.0%	80.0%	443 ± 101	79%	18%	2%	0%	0%	0%	1%
2	20.0%	26.7%	60.0%	170 ± 68	63%	26%	7%	0%	1%	0%	3%
3	13.3%	6.7%	80.0%	443 ± 101	71%	26%	1%	0%	1%	0%	1%
4	13.3%	6.7%	46.7%	47 ± 22	58%	31%	7%	0%	0%	0%	4%
5	46.7%	13.3%	73.3%	673 ± 188	59%	28%	7%	0%	2%	0%	4%
6	13.3%	6.7%	73.3%	397 ± 115	57%	32%	6%	0%	0%	0%	5%
7	28.6%	14.3%	85.7%	475 ± 109	64%	32%	0%	2%	0%	0%	2%
8	6.7%	20.0%	73.3%	823 ± 235	51%	41%	2%	0%	1%	0%	5%
9	46.7%	13.3%	80.0%	723 ± 200	50%	48%	0%	0%	0%	0%	2%
10	28.6%	0.0%	50.0%	393 ± 135	50%	43%	6%	0%	1%	0%	0%
11	13.3%	13.3%	40.0%	57 ± 28	47%	44%	9%	0%	0%	0%	0%
12	23.1%	0.0%	69.2%	404 ± 126	44%	46%	4%	0%	0%	0%	6%
13	6.7%	0.0%	60.0%	60 ± 28	47%	35%	6%	0%	3%	3%	6%
14	20.0%	13.3%	20.0%	20 ± 12	53%	41%	6%	0%	0%	0%	0%
15	53.3%	0.0%	46.7%	40 ± 13	53%	44%	3%	0%	0%	0%	0%
16	7.1%	14.3%	71.4%	586 ± 130	51%	43%	3%	0%	1%	2%	0%

[a] s.e.: standard error of the mean.

Table 3
Results of association of prevalence of subclinical mastitis with parasitic infection measures according to varying thresholds of epg counts in 16 sheep farms in Greece.

Threshold (epg counts)	Prevalence of subclinical mastitis in ewes with		
	epg counts above threshold	epg counts below threshold	*P*
300.0[a]	30.9%	18.8%	0.029
357.7[b]	31.1%	18.6%	0.027
600.0[c]	34.0%	19.0%	0.019
854.4[d]	36.1%	20.1%	0.033

[a] 300.0 epg is the value considered in an earlier study as the threshold for performing targeted anthelmintic treatments in dairy sheep in Greece (Gallidis et al., 2009).
[b] mean epg count in the population sampled in the current study.
[c] twice the value set in the study of Gallidis et al. (2009).
[d] mean epg count in the population sampled in the current study plus one standard deviation.

3.3. Associations between parasitic infections and subclinical mastitis

There was clear evidence that prevalence of subclinical mastitis was higher among ewes with gastrointestinal nematode infection than among non-parasitised ewes: 26.4% for those with gastrointestinal nematode infection versus 16.1% for those with no gastrointestinal nematode infection ($P = 0.047$). The prevalence of subclinical mastitis was higher in ewes with high faecal epg: 18.6% for ewes with epg < 357.7 versus 31.1% for ewes with epg > 357.7 ($P = 0.027$). Also, the prevalence of subclinical mastitis was always higher in ewes with high epg counts than in ewes with low counts, independently of the threshold employed for the analysis ($P < 0.035$ for all comparisons) (Table 3). The trend of prevalence of subclinical mastitis in ewes according to faecal epg counts (in increments of 400 epg) is shown in Fig. 1.

The same trend, i.e., higher prevalence of subclinical mastitis among ewes with gastrointestinal nematode infection and among ewes with epg counts higher than the farm's average, was seen in 12 of the 16 farms, as well as partially in another farm. In 3 farms, prevalence of subclinical mastitis was higher among ewes with no gastrointestinal nematode infection and with low epg counts (Table 4).

There was clear evidence that mean epg counts in ewes with mastitis were significantly higher: 500 ± 84 (mean ± standard error of the mean), than epg counts in healthy ewes: 316 ± 36 ($P = 0.024$) (Fig. 2). However, there was no difference in mean epg counts in ewes with mastitis in relation to causal agent of the disease: 521 ± 121 for ewes with mastitis caused by coagulase-negative staphylococci and 470 ± 112 for ewes with mastitis caused by *S. aureus* or *M. haemolytica* ($P = 0.38$).

Table 4
Detailed results of association of prevalence of subclinical mastitis with parasitic infection measures in 16 sheep farms in Greece.

Farm	Prevalence of subclinical mastitis in ewes with					
	GNI[a]	no GNI[a]	*P*	high epg[b]	low epg[b]	*P*
1	25.0%	0.0%	0.48	25.0%	14.3%	0.55
2	22.2%	16.7%	0.66	100.0%	7.7%	0.12
3	16.7%	0.0%	0.63	25.0%	0.00%	0.27
4	28.6%	0.0%	0.20	0.00%	13.3%	1.00
5	54.5%	25.0%	0.34	62.5%	28.6%	0.21
6	18.2%	0.0%	0.52	40.0%	0.00%	0.09
7	25.0%	50.0%	0.51	28.6%	28.6%	0.72
8	9.1%	0.0%	0.73	12.5%	0.00%	0.53
9	41.7%	66.7%	0.45	37.5%	57.1%	0.40
10	42.9%	14.3%	0.28	16.7%	33.3%	0.46
11	33.3%	0.0%	0.14	0.00%	13.3%	1.00
12	30.0%	0.0%	0.33	33.3%	0.00%	0.19
13	11.1%	0.0%	0.60	0.00%	6.7%	1.00
14	0.0%	33.3%	0.48	0.00%	20.0%	1.00
15	71.4%	37.5%	0.21	0.00%	53.3%	1.00
16	10.0%	0.0%	0.71	11.1%	0.00%	0.64

[a] GNI: gastrointestinal nematode infection.
[b] high / low epg: higher / lower than 357.7 epg.

On average, more isolates were recovered from ewes with high faecal epg (1.39 isolates per ewe with subclinical mastitis) than from ewes with low faecal epg (1.20 isolates per ewe with subclinical mastitis) ($P = 0.13$); there was no trend in differences regarding identity of bacteria isolated from cases of the disease. Bilateral mastitis was evident in 26.1% of ewes with mastitis and high epg counts and 16.7% of ewes with mastitis and low epg counts ($P = 0.31$).

Prevalence of subclinical mastitis in ewes sampled in farms with high mean faecal epg was not significantly higher than in farms with low mean faecal epg: 24.1% versus 21.0%, respectively ($P = 0.34$). Further, no significant difference in prevalence of subclinical mastitis was seen in farms with high *Teladorsagia* proportion in coproculture: 26.1%, *versus* farms with low proportion: 19.0% ($P = 0.13$). Similar

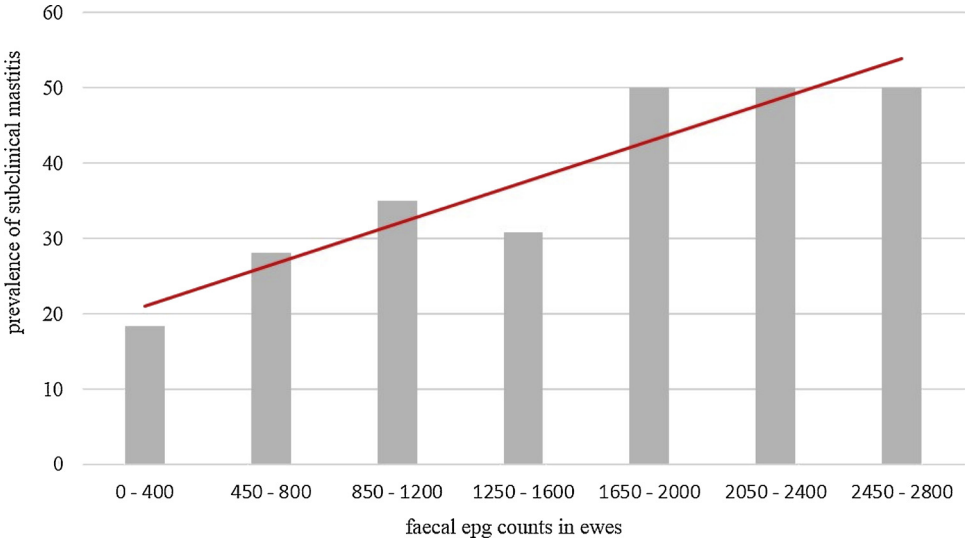

Fig. 1. Prevalence of subclinical mastitis in ewes according to faecal epg counts (in clusters separated by increments of 400 epg); trend line in red colour (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

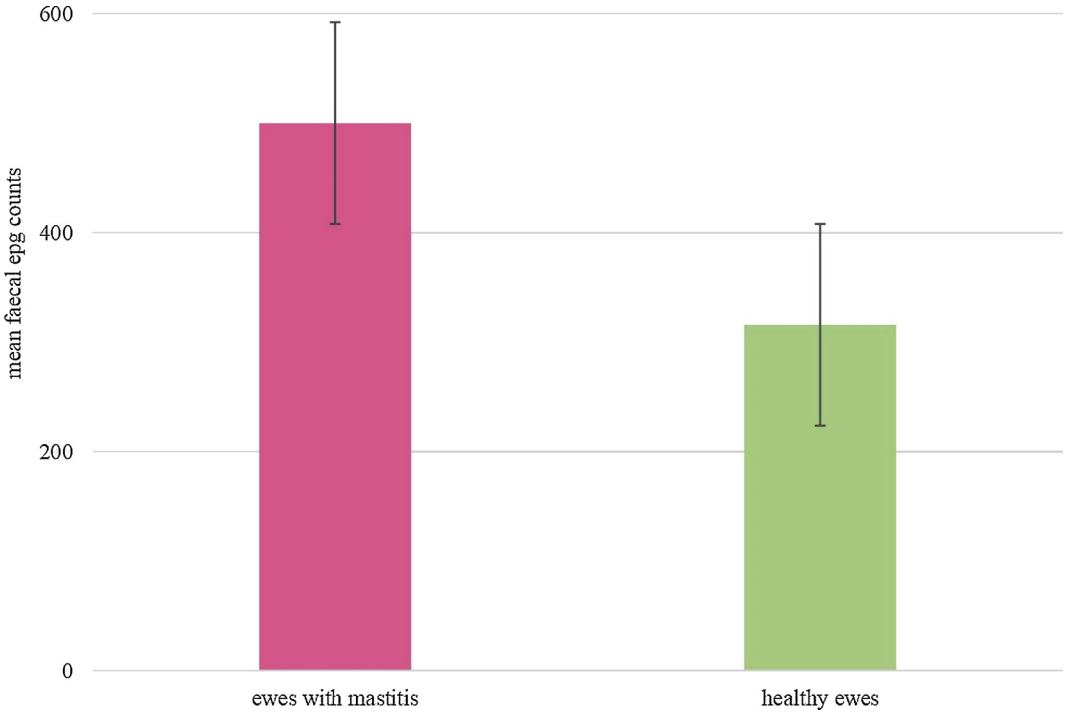

Fig. 2. Mean faecal epg counts in ewes with subclinical mastitis (red bar) or in healthy ewes (green bar) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

results were seen when prevalence of subclinical mastitis in farms with high *Haemonchus* proportion in coproculture: 25.0%, were compared with farms with low proportion: 20.2% ($P = 0.23$). Finally, there was no correlation of mastitis prevalence with mean epg counts in farms ($P = 0.33$).

Regarding mammary carriage, all the above computations were repeated, but no significance was detected ($P > 0.115$).

4. Discussion

Gastrointestinal nematode parasitism is a global problem and is the most frequent infection of sheep (Kaplan and Vidyashankar, 2012; Taylor, 2012). Most often, the infection does not lead to disease, but, nevertheless, adversely affects production of animals (Mavrot et al., 2015). Moreover, gastrointestinal nematode infections may contribute to compromised immune defence of affected sheep by means of various mechanisms, e.g., changes in size and rate of turnover of cell and protein pools of the immune system (Sykes, 2010).

Various factors may predispose sheep to clinical mastitis (Gelasakis et al., 2015; Vasileiou et al., 2018a). In a previous study by Mavrogianni et al. (2017), the role of gastrointestinal nematodes in predisposing ewes to mastitis caused by *M. haemolytica*, was evaluated In that study, which took place in experimental conditions, it was shown that increased parasitic burden contributed to development of clinical mastitis and that mainly *Teladorsagia* spp. infections were predisposing ewes to clinical mastitis.

In the present study, we focused in potential effects of gastrointestinal nematode parasitism in subclinical mastitis. This form of the disease has not been studied as extensively as clinical mastitis and relevant predisposing factors have not been clarified well (Vasileiou et al., 2018a). Moreover, the present study refers to field work, where many aetiological agents of mastitis were implicated. The results suggest that gastrointestinal nematode infections are associated with increased prevalence of subclinical mastitis in ewes. The findings indicated that ewes with high epg counts also had subclinical mastitis more frequently animals with low epg counts. This finding was independent of four thresholds used to cluster animals with high or low epg counts.

The most likely reason for the increased prevalence of mastitis is the compromise in host defences as a consequence of parasitism (Prada Jiménez de Cisneros et al., 2014; McRae et al., 2015). The clear association of high parasitic burdens with mastitis lends support to this argument: prevalence of subclinical mastitis was higher in ewes with epg above the mean count among population into the study. There are various pathways that can be considered. For example, increased numbers of nematode helminths lead in reduced feed intake (Coop and Holmes, 1996), thus contributing in negative energy balance (especially in lactating ewes), which can affect mammary defences, e.g., neutrophil migration and influx into the mammary gland; energy depletion caused by parasitic infections, especially in high-yielding dairy ewes, would also contribute in impediment of mammary defences, e.g., delayed mobilisation to the mammary gland, impaired phagocytosis, inefficient intracellular killing, which would contribute to development of severe disease (Szilard et al., 2002); further, negative energy balance can lead in subclinical ketosis (Karagiannis, 2016), during which there is increased concentration of β-hydroxybutyrate in blood.

Whilst clinical ketosis in lactating ewes is occasional (Jopp and Quinlivan, 1981), subclinical manifestation of the condition occurs frequently in sheep flocks (Karagiannis, 2016), with blood concentrations of β-hydroxybutyrate often reaching levels of up to 3.0 mmol L^{-1} (Karagiannis, 2016). Increased concentrations of β-hydroxybutyrate might impede leucocytic response in cases of bacterial invasion into the mammary gland. Ketonaemic animals may have smaller amounts of interferons than normal animals (Kandefer-Szerszen et al., 1992), reduced quantities of which may inhibit the animal to mount an effective defence response. Suriyasathaporn et al. (1999) reported that leucocytes from ketotic cows had lower chemotactic ability than those from healthy animals. Sartorelli et al. (1999), who worked specifically in ewes, have shown that increased concentrations of β-hydroxybutyrate could adversely affect particle uptake by neutrophils. All above mechanisms contribute to impaired phagocytosis and given the particular significance of effective phagocytosis in mastitis pathogenesis (Fragkou et al., 2017), the adverse effects of increased β-hydroxybutyrate concentrations (even at subclinical concentrations) are obvious.

Moreover, local defences in the teat of parasitised ewes may also be

impaired. This has been clearly shown in experimental models (Mavrogianni et al., 2017); in parasitised ewes, lack of the organised teat defences, which limit bacterial multiplication and prevent invasion of bacteria into the mammary parenchyma (Mavrogianni et al., 2005), increases risk of mastitis development.

In contrast to the above, no association was found between parasitism and mammary carriage. Bacteria can easily colonise the teat duct, but they are limited by effective defences of sheep. Although colonisation of the udder takes place independently of parasitic infection (Mavrogianni et al., 2007), it can then be potentially followed by invasion of the mammary parenchyma and development of mastitis. Fragkou et al. (2007a,b) were the first to indicate that the teat duct flora can become pathogenic and cause mastitis under circumstances leading in reduction of mammary or systemic defences of animals. Lack of association with a particular mammary pathogen, as found in the study, lends further support to this argument. Indeed, mammary defence relies primarily on leucocytes, which provide a non-specific defence mechanism against invading pathogens (Fragkou et al., 2017). In the experimental study by Mavrogianni et al. (2017), we have provided evidence and we have discussed various pathways through which udder defences would be impeded by heavy parasitic infections, thus leading to development of clinical mastitis: the evidence in that study indicated that cellular defences in the udder of infected ewes had been depleted, thus increasing risk for development of clinical mastitis.

During the study, the association of gastrointestinal nematode parasitism with presence of subclinical mastitis has only been identified at animal level and not at farm level. This may be explained by the multi-faceted nature of mastitis. Many factors can act to increase risk of the disease and many other are involved in its pathogenesis (Gelasakis et al., 2015). At farm level, where various factors apply and where udder health management measures are implemented, significance of gastrointestinal parasitism might not be significant for increased risk to mastitis. In contrast, individual animals with decreased defensive abilities (due to parasitism), in which specific factors contributing to mammary infection would occur (e.g., spontaneous cluster drop during milking, failure to provide complete teat cover with disinfectant in teat-dipping, incorrect anti-mastitis vaccination), may be more exposed to bacterial invasion and mastitis. During parasitic infections, the host attempts to elicit an effective immune response to expel the parasite and minimise its harmful effects (Anthony et al., 2007), therefore it may have different responses to bacteria simultaneously invading its tissues.

The findings underline the importance of concurrent parasitic and bacterial infections and provide further evidence that parasitic diseases could lead to aggravated pathogenic action of microorganisms. Moreover, results also indicate the many factors that can play a role in development of mastitis in ewes. In general, management factors are considered as the most significant factors predisposing ewes to mastitis (Vasileiou et al., 2018a). The present results support the hypothesis that various infections may also play a predisposing role for mastitis, even for subclinical infection, which has not been studied extensively thusfar.

The study was performed in dairy sheep, where mastitis has an obvious financial significance (Gelasakis et al., 2015). However, mastitis has been recognised as an important welfare challenge in ewes, independently of production type and management system (European Food Safety Authority, 2014), and occurs also in meat- (Arsenault et al., 2008) and wool- (Omaleki et al., 2010) production flocks. The major features of mastitis are common among dairy and meat-producing sheep. However, there is a well-recognised breed-susceptibility to mastitis (Fragkou et al., 2007a,b; Vasileiou et al., 2018b), with indigenous sheep breeds producing low milk yield considered to be less susceptible to mastitis (Vasileiou et al., 2018b). As there are significant breed differences between dairy and meat-producing sheep, one can consider that present results would not be directly extrapolated to sheep under different production types.

Conflict of interest statement

The authors have nothing to disclose.

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