



Use of geographical information system and ecological niche modelling for predicting potential space distribution of subclinical mastitis in ewes



A. Giannakopoulos^{a,1}, N.G.C. Vasileiou^{a,1}, D.A. Gougoulis^a, P.J. Cripps^b, K.S. Ioannidi^a, D.C. Chatzopoulos^a, C. Billinis^a, V.S. Mavrogianni^a, E. Petinaki^c, G.C. Fthenakis^{a,*}

^a Veterinary Faculty, University of Thessaly, 43100, Karditsa, Greece

^b Institute of Veterinary Science, University of Liverpool, Neston, South Wirral, CH64 7TE, United Kingdom

^c University Hospital of Larissa, 41110, Larissa, Greece

ARTICLE INFO

Keywords:

Climate
Environment
Mastitis
Prediction
Sheep
Subclinical mastitis

ABSTRACT

Objective was to recognise areas potentially of high risk for increased frequency of subclinical mastitis in ewes. Milk samples were collected, for bacteriological and cytological examination, from 2198 clinically healthy ewes in 111 farms in all administrative regions of Greece. Data on farms were located in the field using hand-held Global Positioning System Garmin units. Collected data were analysed by an Ecological Niche Model under the framework of a geographic information system. Two separate analyses were performed: one for subclinical mastitis independently of causal agent (prevalence in population sampled: 0.260) and one for subclinical mastitis caused specifically by slime-producing staphylococci (prevalence in population sampled: 0.153). A model was constructed in which sheep farms were divided into two clusters, according to prevalence of subclinical mastitis: farms in the upper three quartiles of prevalence were used as occurrence points for the Ecological niche modelling procedure ('infected farms'); farms in the lower quartile of prevalence within each category were (pseudo)negative points. Significant differences in environmental parameters prevailing in locations of farms into the study, were identified for up to 13 parameters between locations of farms according to management system applied in farms. When farms in each management system were considered separately, differences became evident between farms in each management system, as well as between the two infections. The factor with the highest relative contribution in the analyses was the distance from other sheep farms; other factors also of importance in the predictive models were the altitude, the maximum temperature of warmest month and the total precipitation of driest month. Verification of the model revealed that ≥ 0.760 of infected farms' were located in areas predicted as high risk for prevalence of subclinical mastitis or slime staphylococcal subclinical mastitis. The paper describes for the first time potential association of mastitis with environmental factors and presents predictive models for mastitis in ewes taking into account environmental parameters.

1. Introduction

Mastitis in ewes is a multi-factorial problem, with many bacteria identified as causal agents and many factors accounting for potential predisposition (Gelasakis et al., 2015; Fthenakis et al., 2017). Mastitis adversely affects production and causes financial problems, especially in dairy flocks, and has also been recognised as the most important cause of welfare concerns in sheep (European Food Safety Authority, 2014).

Possible involvement of climatic conditions in development of mastitis has not been systematically investigated. Climatic factors are of

significance in development of infections, as they can contribute to transmission of pathogens or may exert effects on hosts. There are very few studies that had investigated environmental factors for risk of mastitis. In the past, Clark (1972) has reported that, in Australia, mastitis was occurring more frequently after gusts of cold winds; another possible association between adverse weather and development of mastitis in ewes has also been reported in the 1980s (Anon, 1988).

Various predictive distribution models have been developed and employed for infections. Among them, Ecological Niche Modelling (ENM), e.g., MaxEnt (Phillips et al., 2006), enables description of full distribution range. In the MaxEnt modelling, the pixels of the study area

* Corresponding author.

E-mail address: gcf@vet.uth.gr (G.C. Fthenakis).

¹ these authors have contributed equally and their names are listed alphabetically.

present the area where the distribution of the MaxEnt probability is defined. Pixels with occurrence records constitute the sample points and the features are environmental parameters. MaxEnt methodology requires presence-only data, utilises both continuous and categorical data and includes efficient deterministic algorithms and mathematical definitions (Phillips et al., 2006).

This paper presents application of the ecological niche model in subclinical mastitis in ewes. Objective of the work was to recognise areas potentially of high risk for increased frequency of subclinical mastitis. To the best of our knowledge, this is the first work presenting predictive models for ruminant mastitis taking into account environmental parameters.

2. Materials and methods

2.1. Study overview: sheep farms, samplings, geographical information system, laboratory examinations

Present work follows up from a large countrywide investigation into subclinical mastitis in ewes, where its prevalence and aetiology, as well as various predictors have been identified (Vasileiou et al., 2018b). In total, 111 sheep farms in the 13 administrative regions of Greece were included into the study and visited for collection of samples. Farms were selected by the collaborating veterinarians on convenience basis (willingness of farmers to accept a visit by University personnel for sample collection). The principal investigators (NGCV and GCF) accompanied by an assisting investigator visited all farms for sample collection.

Data on farm location were collected in the field using hand-held Global Positioning System Garmin units. The geo-references were resolved to specific farm level. ArcGIS V.10.1 GIS software (ESRI; Redlands, Ca, USA) was employed for description and analysis of spatial information. In each farm, 20 clinically healthy ewes were sampled. Details of selection of animals for sampling have been presented before (Vasileiou et al., 2018b). Examinations performed in ewes, as well as of sampling procedures followed have been detailed before (Vasileiou et al., 2018b). Conventional microbiological examinations were performed in milk samples collected during the study for bacterial identification (Vasileiou et al., 2018b). Further, cytological examination by the California Mastitis Test and the Microscopic cell counting method were also performed in milk samples (Vasileiou et al., 2018b).

Staphylococcal strains recovered during the study were subsequently tested for *in vitro* slime production, by a combination of culture appearance on Congo Red agar and microplate adhesion method. The methodology of the work and the techniques employed have been detailed by Vasileiou et al. (2018a). In brief, established techniques, previously published (Freeman et al., 1989; Vasudevan et al., 2003; Fabres-Klein et al., 2015), were followed. Finally, strains have been classified as slime-producing, weak slime-producing or non slime-producing by combining results of the two methods (Vasileiou et al., 2018a).

2.2. Data management and analysis

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 (≥ 1) plus neutrophil and lymphocyte proportion ($\geq 65\%$ of all leukocytes) was detected (Fragkou et al., 2014; Vasileiou et al., 2018a; 2018b). Cases of subclinical mastitis found to be caused by staphylococcal strains producing slime were termed 'slime staphylococcal subclinical mastitis'; for that, slime-producing and weak slime-producing strains were taken into account. All above definitions referred to ewes (hence, animals with both glands affected were counted as one case).

Climatic variables (11 related to temperature, 8 related to

Table 1
Prevalence (lower quartile) of subclinical mastitis and slime staphylococcal subclinical mastitis in sheep farms into a countrywide investigation in Greece.

	Prevalence (lower quartile)	
	Subclinical mastitis ^a	Slime staphylococcal subclinical mastitis ^b
All farms (n = 111)	0.260 (0.150)	0.153 (0.050)
Farms under intensive management ¹ (n = 26)	0.254 (0.100)	0.158 (0.038)
Farms under semi-intensive management ¹ (n = 57)	0.296 (0.200)	0.174 (0.100)
Farms under semi-extensive / extensive management ¹ (n = 28)	0.192 (0.063)	0.096 (0.000)

¹ Management systems classified as per European Food Safety Authority (2014).

^a $P < 0.001$ between management systems.

^b $P = 0.004$ between management systems.

precipitation, all with continuous values) were derived from the WorldClim website (<http://www.worldclim.org>), version 1.4. (Hijmans et al., 2005; WorldClim – Global Climate Data, 2017). ArcGIS 10.1 GIS software (ESRI, Redlands, CA, USA) was used to create environmental layers (n = 19) (*clima1*, ..., *clima19*) for the analysis. Altitude was extracted from a digital elevation model (*dem*) with a spatial resolution of 1 square kilometer, presented in the CGIAR Consortium for Spatial Information (2018). Distances between small ruminant farms (*farmsdis*) were taken from Greek public authority sources, specifically the Greek Payment and Control Agency for Guidance and Guarantee Community Aid (2018) and the Hellenic Statistical Authority (2018). These data sets were converted to a common projection, map extent and resolution prior to use in the modelling program. Comparisons between environmental parameters prevailing in the three categories of farms were performed by using analysis of variance. Statistical significance was defined at $P < 0.05$.

Two separate analyses were performed: one for subclinical mastitis independently of causal agent, and one for slime staphylococcal subclinical mastitis. For each analysis, initially all farms into the study (n = 111) were taken into account; this was followed by taking into account results of farms following the intensive (n = 26), semi-intensive (n = 57) or semi-extensive / extensive (n = 28) management system separately. Classification of farms into management system was performed according to the criteria of the European Food Safety Authority (European Food Safety Authority, 2014). Management system in flocks has been previously identified from multivariable mixed-effects model analyses applied in results of the study, as a significant predictor for subclinical mastitis and slime staphylococcal subclinical mastitis (Vasileiou et al., 2018a; 2018b).

Within each category, farms were divided into two clusters, according to prevalence of subclinical mastitis. Sheep farms classified in the upper three quartiles of prevalence (in analysis for subclinical mastitis: n = 78 [all farms in the study], n = 19 [farms in intensive management], n = 38 [farms in semi-intensive management], n = 21 [farms in semi-extensive / extensive management system] – in analysis for subclinical mastitis: n = 75, n = 20, n = 31, n = 18, respectively) were used as occurrence points for the Ecological niche modelling procedure ('infected farms'); farms classified in the lower quartile of prevalence within each of above categories were used as (pseudo)negative points. Maximum entropy modelling (MaxEnt software ver. 3.3.3) was used to predict the appropriate ecological niches for infected farms (Phillips et al., 2006). The 'bias file' was included in the analysis, in order to represent the sampling effort and to reduce sampling bias. The goodness of fit of the model predictions was evaluated by the mean area under the curve (AUC) of the receiver operating characteristic curve (ROC). The Jackknife procedure was used to reduce the number

Table 2
Environmental conditions (mean \pm standard error of the mean) prevailing in sheep farms into a countrywide investigation in Greece.

Code	Environmental variable	Farm management system ¹		
		Intensive (n = 26)	Semi-intensive (n = 57)	Semi-extensive / extensive (n = 28)
<i>clima1</i>	Annual mean temperature (°C)	15.4 \pm 0.2 ^A	15.9 \pm 0.2 ^B	16.8 \pm 0.3 ^{A,B}
<i>clima2</i>	Mean diurnal temperature range (°C)	10.9 \pm 0.2 ^A	10.1 \pm 0.2 ^B	8.7 \pm 0.4 ^{A,B}
<i>clima3</i>	Isothermality (<i>clima2</i> / <i>clima7</i> \times 100)	35.2 \pm 0.4 ^A	34.5 \pm 0.3 ^a	33.0 \pm 0.3 ^{a,A}
<i>clima4</i>	Temperature seasonality (°C)	6799 \pm 128 ^A	6553 \pm 97 ^a	6122 \pm 141 ^{a,A}
<i>clima5</i>	Maximum temperature of warmest month (°C)	32.2 \pm 0.4	31.8 \pm 0.3	30.8 \pm 0.3
<i>clima6</i>	Minimum temperature of coldest month (°C)	1.8 \pm 0.3 ^A	3.0 \pm 0.4 ^B	5.1 \pm 0.6 ^{A,B}
<i>clima7</i>	Temperature annual range (°C)	30.4 \pm 0.6 ^A	28.9 \pm 0.5 ^B	25.8 \pm 0.8 ^{A,B}
<i>clima8</i>	Mean temperature of wettest quarter (°C)	9.0 \pm 0.4 ^a	9.6 \pm 0.3	10.5 \pm 0.4 ^a
<i>clima9</i>	Mean temperature of driest quarter (°C)	23.9 \pm 0.2	24.2 \pm 0.2	24.7 \pm 0.2
<i>clima10</i>	Mean temperature of warmest quarter (°C)	15.4 \pm 0.2 ^A	15.9 \pm 0.2 ^a	16.8 \pm 0.3 ^{a,A}
<i>clima11</i>	Mean temperature of coldest quarter (°C)	6.9 \pm 0.3 ^A	7.8 \pm 0.3 ^B	9.3 \pm 0.4 ^{A,B}
<i>clima12</i>	Total annual precipitation (mm)	643 \pm 30	654 \pm 19	651 \pm 29
<i>clima13</i>	Total precipitation of wettest month (mm)	98 \pm 7	106 \pm 4	121 \pm 9
<i>clima14</i>	Total precipitation of driest month (mm)	14 \pm 1 ^A	12 \pm 1 ^B	7 \pm 1 ^{A,B}
<i>clima15</i>	Precipitation seasonality (%)	46.4 \pm 2.9 ^A	53.5 \pm 2.5 ^B	66.6 \pm 3.8 ^{A,B}
<i>clima16</i>	Total precipitation of wettest quarter (mm)	263 \pm 18	282 \pm 12	318 \pm 23
<i>clima17</i>	Total precipitation of driest quarter (mm)	60 \pm 3 ^A	56 \pm 4 ^B	36 \pm 5 ^{A,B}
<i>clima18</i>	Total precipitation of warmest quarter (mm)	64 \pm 4 ^A	12 \pm 1 ^B	7 \pm 1 ^{A,B}
<i>clima19</i>	Total precipitation of coldest quarter (mm)	244 \pm 18	261 \pm 11	289 \pm 20

^{a,b} between columns: $0.01 < P \leq 0.04$, ^{A,B} between columns: $P < 0.01$.

¹ Management systems classified as per European Food Safety Authority (2014).

Table 3
Estimation of relative contributions of the variables to the MaxEnt model for subclinical mastitis and slime staphylococcal subclinical mastitis in all sheep farms into a countrywide investigation in Greece.

Code	Environmental variable	Subclinical mastitis		Slime staphylococcal subclinical mastitis	
		% contribution	Permutation importance	% contribution	Permutation importance
<i>clima1</i>	Annual mean temperature	1.2	1.2	0.2	0.0
<i>clima2</i>	Mean diurnal temperature range	0.3	0.0	0.2	0.7
<i>clima3</i>	Isothermality	0.0	0.1	0.7	1.4
<i>clima4</i>	Temperature seasonality	2.4	1.6	3.0	1.0
<i>clima5</i>	Maximum temperature of warmest month	22.1	16.5	15.9	23.0
<i>clima6</i>	Minimum temperature of coldest month	0.0	0.0	0.0	0.0
<i>clima7</i>	Temperature annual range	1.1	0.4	0.3	0.2
<i>clima8</i>	Mean temperature of wettest quarter	0.0	0.3	0.0	0.2
<i>clima9</i>	Mean temperature of driest quarter	1.9	2.6	3.0	2.3
<i>clima10</i>	Mean temperature of warmest quarter	0.0	0.1	0.0	0.0
<i>clima11</i>	Mean temperature of coldest quarter	0.0	0.0	0.0	0.0
<i>clima12</i>	Total annual precipitation	1.2	1.4	0.8	1.0
<i>clima13</i>	Total precipitation of wettest month	3.1	4.1	3.6	2.8
<i>clima14</i>	Total precipitation of driest month	2.9	8.7	2.7	8.7
<i>clima15</i>	Precipitation seasonality	0.1	0.2	0.0	0.7
<i>clima16</i>	Total precipitation of wettest quarter	0.0	0.0	0.0	0.0
<i>clima17</i>	Total precipitation of driest quarter	0.0	0.0	0.0	0.0
<i>clima18</i>	Total precipitation of warmest quarter	0.1	1.3	0.0	0.0
<i>clima19</i>	Total precipitation of coldest quarter	0.1	0.0	0.1	0.8
<i>farmsdis</i>	Distance from sheep farms	55.7	51.4	59.3	54.8
<i>dem</i>	Altitude	7.8	10.1	10.1	2.4

of environmental variables to only those that showed a substantial influence on the model.

After completion of the analysis and construction of the predictive maps, the model was verified by checking which of the ‘infected farms’ were indeed located in areas predicted as high risk for prevalence of subclinical mastitis.

3. Results

3.1. Prevalence of subclinical mastitis

In the 111 farms, there were 35,925 ewes. In total, 2198 ewes with clinically normal udders were sampled. Among these, 572 ewes were detected with subclinical mastitis; its prevalence in the population sampled was 0.260 (95% confidence intervals [C.I.]: 0.242 - 0.279).

Further, 337 ewes were detected with slime staphylococcal subclinical mastitis; its prevalence in the population sampled was 0.153 (95% C.I.: 0.139 - 0.169). Further details are in Table 1.

3.2. Environmental parameters

Significant differences ($P \leq 0.04$) in environmental parameters prevailing in locations of farms into the study, were identified for 13 parameters between locations of farms with intensive management and farms with semi-extensive / extensive management and for 11 parameters between locations of farms with semi-intensive management and farms with semi-extensive / extensive management. No differences ($P > 0.13$) were evident for any parameter between locations of farms with intensive management and farms with semi-intensive management. Results are in Table 2. Further, no differences were seen in

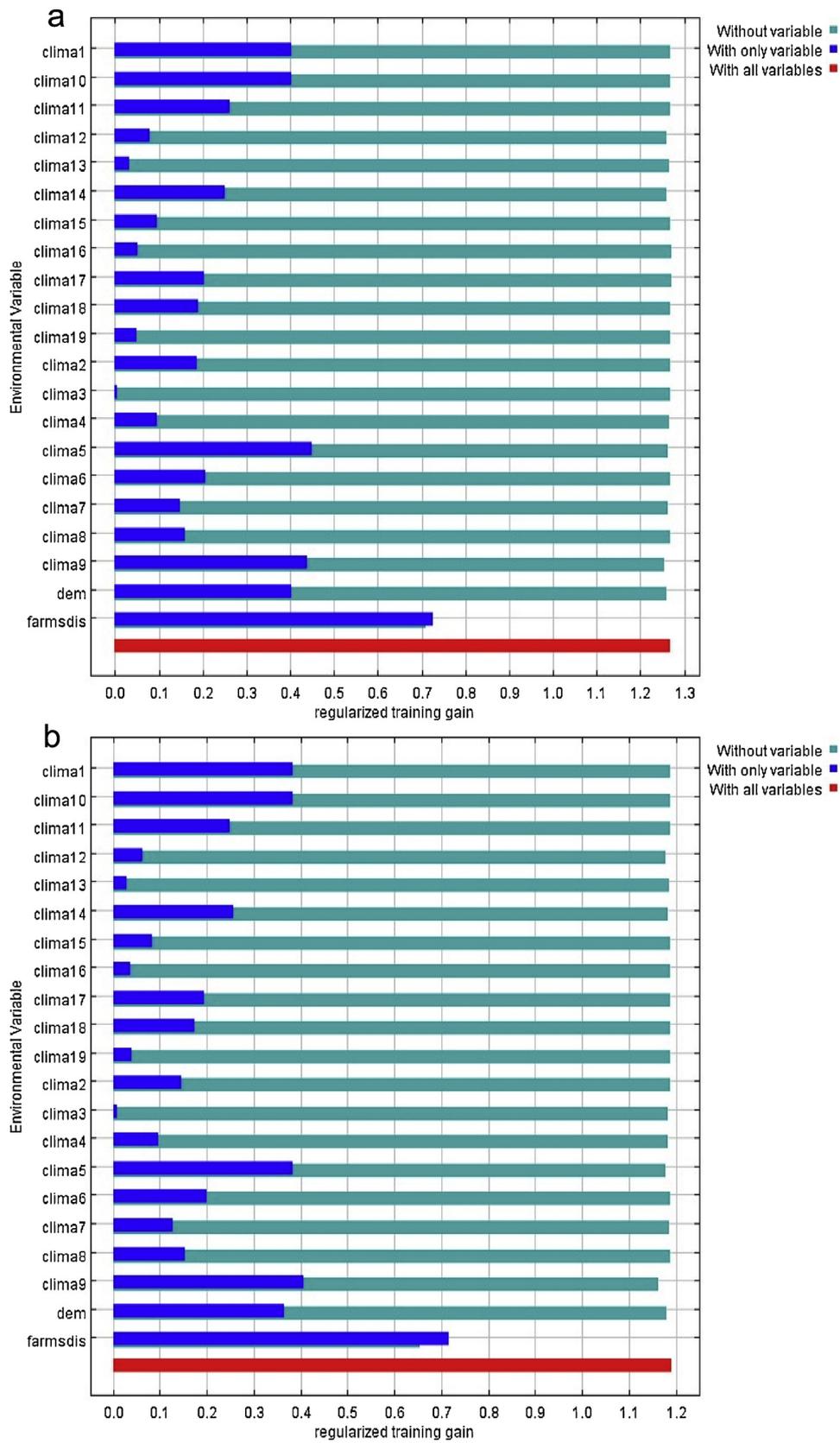


Fig. 1. Jackknife of regularised training gain test for all farms in relation to prevalence of (a) subclinical mastitis or (b) slime staphylococcal subclinical mastitis in sheep in Greece.

Table 4

Estimation of the relative contributions of the variables to the MaxEnt model for subclinical mastitis and slime staphylococcal subclinical mastitis, according to management system in sheep farms into a countrywide investigation in Greece.

Subclinical mastitis						
Environmental variable	Intensive management		Semi-intensive management		Semi-extensive / extensive management	
	% contribution	Permutation importance	% contribution	Permutation importance	% contribution	Permutation importance
Distance from sheep farms	41.8	28.0	56.3	48.0	44.6	34.7
Altitude	11.6	30.4	4.8	4.3	11.1	18.5
Maximum temperature of warmest month	24.7	0.0	29.8	15.3	0.0	0.0
Total precipitation of driest month	8.5	25.6	0.0	0.2	6.9	28.0
Annual mean temperature	6	0.9	0.6	2.6	12.6	0
Mean temperature of driest quarter	0.3	0.0	0.3	1.3	12.9	4.8
Total precipitation of coldest quarter	0.0	0.0	2.4	12.3	1.2	0.0
Minimum temperature of coldest month	1.3	9.4	0.2	1.0	0.1	0.0
Mean diurnal temperature range	1.4	4.2	1.8	3.9	0.0	0.5

Slime staphylococcal subclinical mastitis						
Environmental variable	Intensive management		Semi-intensive management		Semi-extensive / extensive management	
	% contribution	Permutation importance	% contribution	Permutation importance	% contribution	Permutation importance
Distance from sheep farms	44.7	27.8	59.6	58.2	41.5	31.1
Altitude	20.2	43.4	5.1	18.4	16.2	11.2
Total precipitation of driest month	1.8	18.4	2.3	2.7	10.2	41.3
Maximum temperature of warmest month	12.9	0.0	25.6	4.5	0.3	1.4
Total precipitation of wettest quarter	0.0	0.0	0.2	0.1	6.2	6
Mean diurnal temperature range	9.5	1.5	0.5	0.8	0.0	0.0
Mean temperature of warmest quarter	1.3	0.0	0.0	0.0	8.2	0.1
Total annual precipitation	0.2	0.0	0.0	0.0	8.1	0.8
Temperature seasonality	0.9	0.3	2.4	3.7	0.0	0.0
Annual mean temperature	5.8	0.0	0.0	0.0	0.8	0.2
Total precipitation of coldest quarter	0.0	0.0	1.7	3.6	0.0	0.0

altitude (194 ± 30 m, 190 ± 22 m, 151 ± 26 m for farms with intensive, semi-intensive or semi-extensive / extensive management, respectively) or distance between sheep farms (328 ± 39 , 313 ± 26 , 370 ± 50 m, respectively) ($P > 0.085$).

3.3. Predictive ecological niche models

In both analyses, with all farms considered in the analysis, the variable with highest gain when used in isolation, was distance between sheep farms, which appeared to have the most useful information by itself; the same variable was also the one that decreased gain the most when omitted. Also in both analyses, other variables with increased gain when used in isolation, were maximum temperature of warmest month, altitude and total precipitation of wettest month. Other variables that decreased gain when omitted, were maximum temperature of warmest month, as well as altitude and total precipitation of driest month (subclinical mastitis) and total precipitation of driest month and mean temperature of driest quarter (slime subclinical mastitis). The contribution of the variables studied in the MaxEnt model for all farms in the study, is in Table 3. Jackknife of regularised training gain test for subclinical mastitis and slime staphylococcal subclinical mastitis in these farms is in Fig. 1. Regularised training gain (sum of the likelihood of the data plus a penalty function) was 1.269 and 1.190, training AUC was 0.906 and 0.905 and unregularised training gain was 1.469 and 1.407, for subclinical mastitis and slime staphylococcal subclinical mastitis, respectively.

Verification of the model indicated that 61 of 78 (0.782) and 66 of 75 (0.880) 'infected farms' were located in areas predicted as high risk for prevalence of subclinical mastitis or slime staphylococcal subclinical mastitis, respectively.

When farms in each management system were considered separately, differences became evident between farms in each management system, as well as between the two infections. Factors with highest relative contribution in each analysis, for farms under intensive, semi-

intensive or semi-extensive / extensive management system were, respectively: maximum temperature of warmest month (in both analyses), total precipitation of driest month and maximum temperature of warmest month (for each of the two analyses) and mean temperature of driest quarter (in both analyses). Relative contributions of the variables studied to the MaxEnt model for sheep farms in each management system are in Table 4. Jackknife of regularised training gain test for subclinical mastitis in these farms are in Fig. 2. Environmental variables with highest gain when used in isolation, in each analysis, for farms under intensive, semi-intensive or semi-extensive / extensive management system were, respectively: maximum temperature of warmest month and annual mean temperature (for each of the two analyses), annual mean temperature and maximum temperature of warmest month (for each of the two analyses) and mean temperature of driest quarter (in both analyses). Details are in Table 5 (Fig. 3).

Verification of the model indicated that, in total, 61 of 78 (0.782) and 62 of 75 (0.827) 'infected farms' were located in areas predicted as high risk for prevalence of subclinical mastitis and slime staphylococcal subclinical mastitis, respectively (Table 5).

4. Discussion

The paper describes, to the best of our knowledge for the first time, potential association of mastitis with environmental factors. Ecological niche modelling has never been used in the study of ruminant mastitis. Further, the methodology has been rarely used in the study of sheep infections. Recently, Valiakos et al. (2017) have reported identification of potential spread of *Coxiella burnetii* infection in central Greece. In earlier studies, Rose and Wall (2011) have employed the methodology to report potential spread of *Lucilia* flies, the causative agent of myiasis, in Great Britain, whilst Kantzoura et al. (2011) have identified increased risk locations for development of fasciolosis in south-east Europe.

Use of ENM has been shown repeatedly to be effective in locating

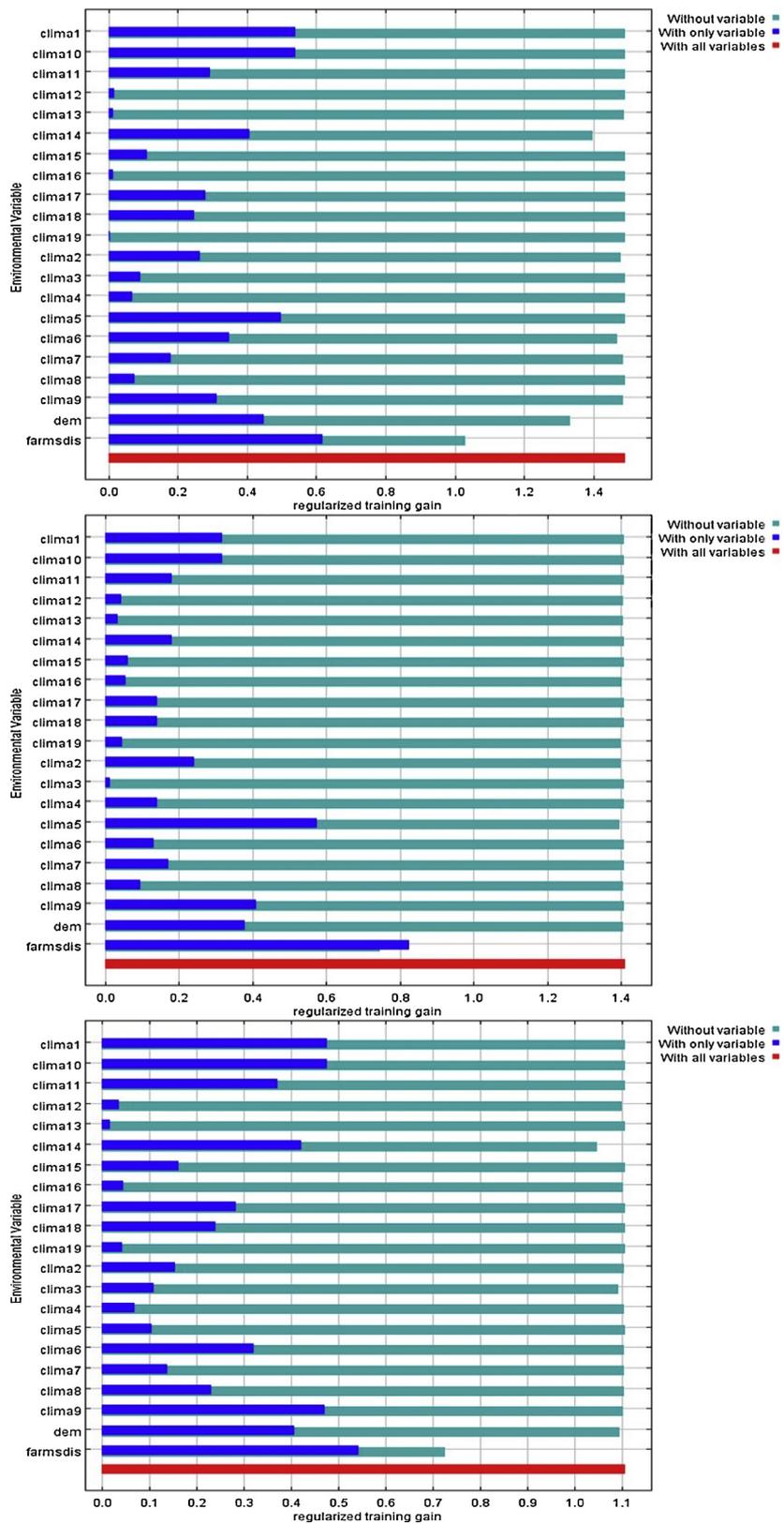


Fig. 2. Jackknife of regularised training gain test for farms in relation to prevalence of subclinical mastitis in ewes in Greece. From top to bottom: Sheep farms with intensive management system; Sheep farms with semi-intensive management system; Sheep farms with semi-extensive / extensive management system.

Table 5
 Details of variables with highest gain and with most decreasing gain, as well as verification outcomes for subclinical mastitis and slime staphylococcal subclinical mastitis, according to management system in sheep farms into a countrywide investigation in Greece.

Environmental variable	Farm management ¹								
	Intensive		Semi-intensive		Semi-extensive / extensive				
	Subclinical mastitis	Slime staphylococcal subclinical mastitis	Subclinical mastitis	Slime staphylococcal subclinical mastitis	Subclinical mastitis	Slime staphylococcal subclinical mastitis	Subclinical mastitis	Slime staphylococcal subclinical mastitis	
Environmental variables with highest gain when used in isolation	Distance from sheep farms	Distance from sheep farms	Distance from sheep farms	Distance from sheep farms	Distance from sheep farms	Distance from sheep farms	Distance from sheep farms	Distance from sheep farms	Distance from sheep farms
Environmental variables that decreased gain the most when omitted	Altitude	Altitude	Distance from sheep farms	Distance from sheep farms	Distance from sheep farms	Distance from sheep farms	Distance from sheep farms	Distance from sheep farms	Altitude nb
Regularised training gain	1.490	1.489	1.410	1.474	1.106	1.030	1.106	1.030	1.030
Training AUC	0.948	0.953	0.930	0.939	0.918	0.922	0.918	0.922	0.922
Unregularised training gain	1.920	1.975	1.744	1.855	1.522	1.478	1.522	1.478	1.478
Verification results ²	0.842	0.900	0.763	0.871	0.762	0.944	0.762	0.944	0.944

¹ Management systems classified as per European Food Safety Authority (2014).

² Proportion of 'infected farms' located in areas predicted as high risk for prevalence of subclinical mastitis or slime staphylococcal subclinical mastitis.

potentially increased risk areas for an infection, as well as to identify environmental / ecological conditions under which it would most likely develop (Peterson et al., 2004; Neerincx et al., 2008; Williams et al., 2009; Donalisio and Peterson, 2011; Ellis et al., 2012). A significant aspect of infection dynamics taken into account by the methodology is that environmental conditions can affect its occurrence by acting on all its components: transmission and pathogenesis systems (e.g., virulence and transmission of organisms, susceptibility of hosts). Therefore, by using ENM methodologies, one might be able to locate areas, where sheep can be at higher risk of an infection.

Among the various ecological niche techniques currently available, MaxEnt is considered to use the best algorithm, thus providing the best predictive models (Elith et al., 2006; Zeimes et al., 2012). MaxEnt software has the advantage that it requires only presence data and small number of occurrences. The program can take into account continuous and categorical predictor variables and also includes a regularisation protocol to protect against overfitting; the methodology, in general, shows very good predictive performance.

Areas showing probability over 65% for presence of subclinical mastitis, as shown by the MaxEnt modelling, can be considered to be high risk areas. Models used in the present study have recognised such areas, which were found to be distributed in locations of small or medium altitudes with agricultural activities. Other high risk areas were located along the coastal areas of the country, in the western and eastern areas of the country. The greatest proportion of high risk areas was found in low altitude (< 300 m) and in irrigated, cultivated agricultural areas or in pastures and agroforestry formations.

During analysis of data of the primary field investigation, it emerged in the multivariable analysis that 'flock management system' was a significant factor affecting prevalence of the infection (Vasileiou et al., 2018b). 'Management system' encompasses various aspects of flock husbandry and organisation, e.g., milking routine, animal breed, housing facilities, nutrition, which have been considered to affect development of mastitis in ewes (Gelasakis et al., 2015; Fthenakis et al., 2017). These, to varying extent each one, contribute to mastitis.

In view of that finding, it has been decided to separate farms into the study in categories according to management system, i.e., under intensive, semi-intensive or semi-extensive / extensive management system. That way the primary factor responsible to predisposing ewes to mastitis had been eliminated, therefore potential effects of environmental factors would have become more pronounced. It is interesting that different environmental factors were identified to be of importance in flocks under the various management systems. Obviously, when the 'management system' had been removed, effects of environmental factors came out more clearly.

The results of the two analyses, when all farms had been taken into account (n = 111), did not reveal noteworthy differences between the two infections in environmental factors for inclusion in the predictive model. Further, when computations were performed separately for each management system, some differences in factors into the predictive model emerged between the two infections, but these did not appear to be striking.

Differences between the two infections point out to a possible effect of climatological factors in the causal bacteria. There is some indication that high temperatures favour growth and survival of slime-producing staphylococcal strains (Martin et al., 2016) in dairy farms, thus may potentially influence virulence of these organisms and development of mastitis according to climatological circumstances.

Staphylococci are transmitted to sheep to cause mastitis from hands of milkers (Marco Melero, 1994) and hand-milking has been identified as a risk factor for mastitis in sheep caused by slime-producing staphylococci (Vasileiou et al., 2018a). The identification of distance from other sheep farms as an important factor in the predictive models, indicates that contact with other sheep populations can contribute to dissemination of pathogens, e.g., through staff visits to farms or movement of animals between farms. The findings of the present study

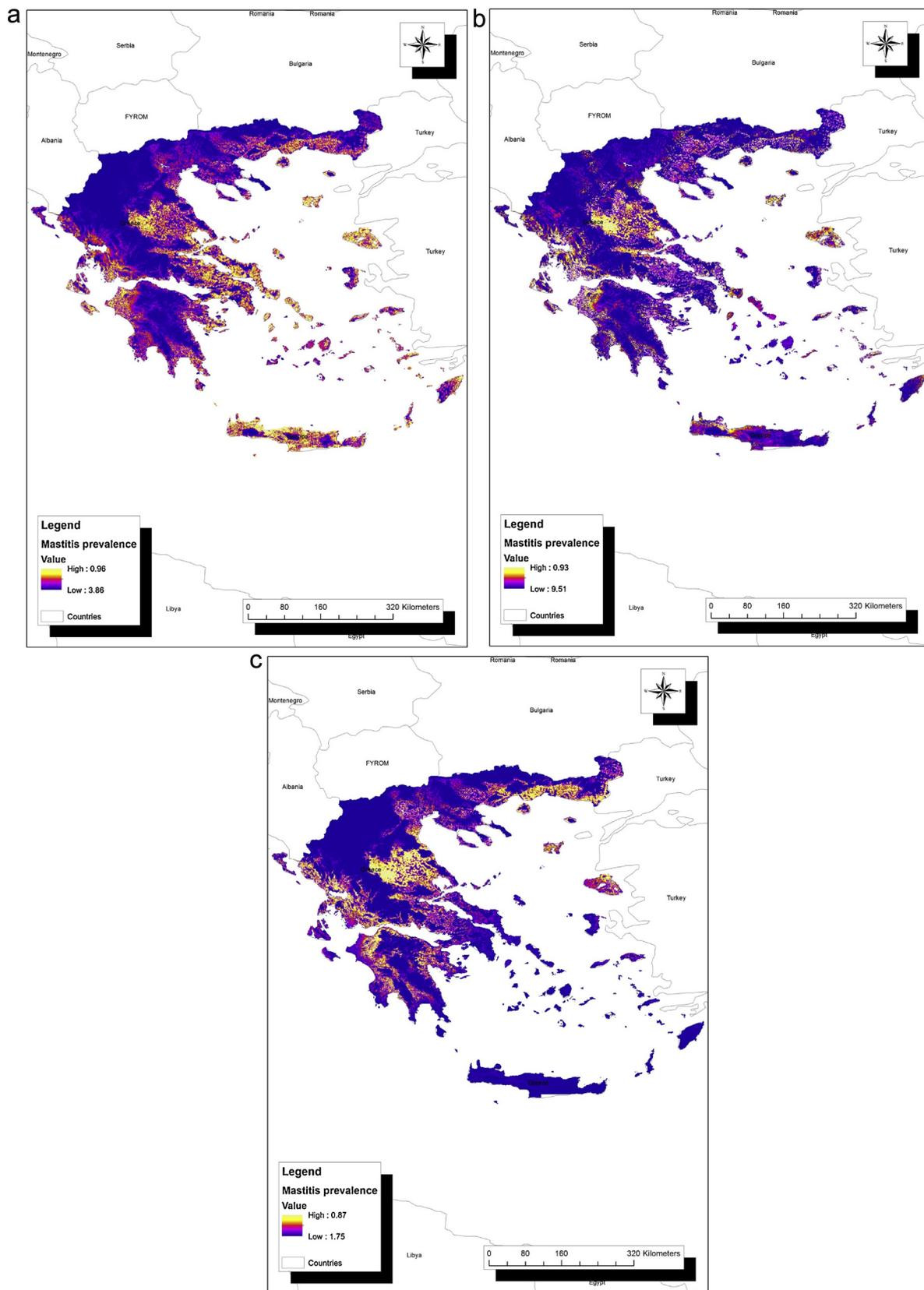


Fig. 3. Maps of Greece showing the potential location of areas of high risk for subclinical mastitis in sheep farms; (a) farms with intensive management, (b) farms with semi-intensive management, (c) farms with extensive / semi-extensive management.

indicate that in sheep (as already well-documented in cattle) there can be between-animal transmission and sheep populations remain a significant risk factor for prevalence of the disease.

Environmental conditions can also affect susceptible hosts and also contribute to development of an infection. High environmental temperatures can lead in reduced leucocyte counts in sheep (El-Tarabany et al., 2017). In such cases, leucocytes have impaired function (e.g., Lacetera et al., 2005; Lecchi et al., 2016). As leucocytes play a significant role in protecting ewes against mastitis, their reduced number and inefficiency would account for the increased prevalence in farms with extreme temperature measurements. Indeed, there is some evidence indicating that in increased temperatures cows develop mastitis more frequently (Arcaro et al., 2013). Moreover, an indirect effect of other factors associated with increased environmental temperatures, e.g., decreased feed intake (Al-Dawood, 2017), which would limit energy availability to defence systems, thus predisposing to mastitis (Barbagianni et al., 2015), should not be ruled out. In contrast, exposure to low temperatures would support increased bacterial colonisation on teat skin, which might subsequently lead to increased bacterial entrance to teat duct and mastitis development (Fox and Norrell, 1994; Fragkou et al., 2007).

Precipitation has not been associated with mastitis before. Only Raboison et al. (2012) have provided an indirect link, when they indicated that increased number of rain days in a month were related to increased somatic cell counts in monthly counts in bulk milk of cows. In the present situation, one may suggest that increased rainfall would lead to muddy pastures, which limit grazing of animals and thus modify feeding patterns, or to low temperatures, which lead to increased energy requirements of animals affecting their immune status (Caroprese et al., 2015).

Moreover, environmental conditions are important with regard to effects in land use and outcomes, which are strongly influenced by temperature and precipitation conditions. Especially in grazing animals, these can affect nutrition of animals, which is a documented predisposing factor for mastitis in ewes (Giadinis et al., 2011; Barbagianni et al., 2015), mainly through regulation of immune response of animal. Farms near arable lands have ready access to quality feedstuffs, whilst in semi-extensive / extensive management systems pasture availability is particularly important to covering nutritional requirements. The significance of altitude as an important factor in the predictive models can be related to the variations in temperature as altitude changes, given that temperature has been identified to be of importance. Moreover, altitude can also be related to the differences in land use, as flora varies with changes in altitude, which can affect grazing of sheep.

Despite environmental factors being outside of the control of farm managers, the present results can be valuable in establishing principles of udder health management in sheep flocks. Hence, in areas with increased risk for mastitis, as indicated herein, relevant preventive measures for mastitis need to be established and applied appropriately, e.g., teat-dipping, which has been found to be an important preventive procedure for mastitis in ewes (Vasileiou et al., 2018b), should be performed. In areas with increased risk of slime staphylococcal subclinical mastitis, measures specifically against these organisms should be applied, e.g., milkers must follow detailed rules in handling animals (e.g., meticulous hand cleaning, glove wearing), given that hand-milking has been associated with increased prevalence of that infection (Vasileiou et al., 2018a); further, milking systems should be thoroughly washed and sanitised as part of the routine applied in milking parlours to reduce populations of slime producing staphylococci in the equipment (Pantoja et al., 2011; Simoes et al., 2015). Finally, during periods when environmental conditions are similar to those leading to high risk for mastitis development, preventive measures should be applied meticulously to minimise risk of mammary infections.

5. Concluding remarks

The above findings have provided, for the first time, predictive models for subclinical mastitis in ewes taking into account environmental (climatic and location) parameters. The findings can be of help for further studies in the field of mastitis and other infections in general, where very little work has been performed with Ecological Niche Modelling. The findings also can be used in mastitis prevention schemes, in order to establish strategies that will help to control mastitis, tailored according to needs of regions and farms, as per environmental risks. Finally, the findings further underline the multifactorial nature of ovine mastitis and indicate additional factors that may play a role in its development.

Declarations of interest

None

Funding sources

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

- Al-Dawood, A., 2017. Towards heat stress management in small ruminants – a review. *Ann. Anim. Sci.* 17, 59–88.
- Anon, 1988. Weather encourages mastitis and pneumonia. *Vet. Rec.* 122, 429–430.
- Arcaro, J.R.P., Matarazzo, S.V., Pozzi, C.R., Arcaro, J.I., de Toledo, L., Costa, E.O., de Miranda, M.S., 2013. Effects of environmental modification on mastitis occurrence and hormonal changes in Holstein cows. *Pesq. Veter. Brasil.* 33, 826–830.
- Barbagianni, M.S., Mavrogianni, V.S., Katsafadou, A.I., Spanos, S.A., Tsioli, V., Galatos, A.D., Nakou, M., Valasi, I., Gouletsou, P.G., Fthenakis, G.C., 2015. Pregnancy toxæmia as predisposing factor for development of mastitis in sheep during the immediately post-partum period. *Small Rumin. Res.* 130, 246–251.
- Caroprese, M., Giannenas, I., Fthenakis, G.C., 2015. Interactions between nutritional approaches and defences against microbial diseases in small ruminants. *Vet. Microbiol.* 181, 8–14.
- CGIAR Consortium for Spatial Information, 2018. <http://srtm.csi.cgiar.org/Index.asp>, (Accessed 12 November 2018).
- Clark, R.G., 1972. Field observations on ovine mastitis. *Proc. 2nd Semin. New Zealand Vet. Assoc. Sheep and Beef Cattle Soc.* 47–54.
- Donalisio, M.R., Peterson, A.T., 2011. Environmental factors affecting transmission risk for hantaviruses in forested portions of southern Brazil. *Acta Trop.* 119, 125–130.
- El-Tarabany, M.S., El-Tarabany, A.A., Atta, M.A., 2017. Physiological and lactation responses of Egyptian dairy Baladi goats to natural thermal stress under subtropical environmental conditions. *Int. J. Biometeorol.* 61, 61–68.
- Elith, J., Graham, C.H., Anderson, R.P., Dudik, M., Ferrier, S., Guisan, A., Hijmans, R., Huettmann, F., Leathwick, J.R., Lehmann, A., Li, J., Lohmann, L.G., Loiselle, B.A., Manion, G., Moritz, C., Nakamura, M., Nakazawa, Y., Overton, J.M.C.M., Townsend Peterson, A., Phillips, S.J., Richardson, K., Scachetti-Pereira, R., Scharipe, R.E., Soberon, J., Williams, S., Wisz, M.S., Zimmermann, N.E., 2006. Novel methods improve prediction of species' distributions from occurrence data. *Ecography* 29, 129–151.
- Ellis, C.K., Carroll, D.S., Lash, R.R., Peterson, A.T., Damon, I.K., Malekani, J., Formenty, P., 2012. Ecology and geography of human monkey pox case occurrences across Africa. *J. Wildl. Dis.* 48, 335–347.
- European Food Safety Authority, 2014. Scientific opinion on the welfare risks related to the farming of sheep for wool, meat and milk production. *EFSA J.* 12, 3933–4060.
- Fabres-Klein, M.H., Santos, M.J.C., Klein, R.C., de Souza, G.N., Ribon, A.D.B., 2015. An association between milk and slime increases biofilm production by bovine *Staphylococcus aureus*. *BMC Vet. Res.* 11, 3.
- Fox, L.K., Norrell, R.J., 1994. *Staphylococcus aureus* colonization of teat skin as affected by post milking teat treatment when exposed to cold and windy conditions. *J. Dairy Sci.* 77, 2281–2288.
- Fragkou, I.A., Boscós, C.M., Fthenakis, G.C., 2014. Diagnosis of clinical or subclinical mastitis in ewes. *Small Rumin. Res.* 118, 86–92.
- Fragkou, I.A., Papaioannou, N., Cripps, P.J., Boscós, C.M., Fthenakis, G.C., 2007. Teat lesions predispose to invasion of the ovine mammary gland by *Mannheimia haemolytica*. *J. Comp. Pathol.* 137, 239–244.
- Freeman, D.J., Falkiner, F.R., Keane, C.T., 1989. New method of detecting slime production by coagulase negative staphylococci. *J. Clin. Pathol.* 42, 872–874.
- Fthenakis, G.C., Barbagianni, M.S., Fragkou, I.A., Gougoulis, D.A., Katsafadou, A.I., Mavrogianni, V.S., Petridis, I.G., Vasileiou, N.G.C., 2017. Elucidation of predisposing factors for ovine mastitis contributes to sustainable control of the disease. *Proc. 9th Int. Sheep Vet. Congr.* 60.
- Gelasakis, A.I., Mavrogianni, V.S., Petridis, I.G., Vasileiou, N.G.C., Fthenakis, G.C., 2015. Mastitis in sheep – the last 10 years and the future of research. *Vet. Microbiol.* 185,

- 136–146.
- Giadinis, N.D., Panousis, N., Petridou, E.J., Siarkou, V.I., Lafi, S.Q., Pourliotis, K., Hatzopoulou, E., Fthenakis, G.C., 2011. Selenium, vitamin E and vitamin A blood concentrations in dairy sheep flocks with increased or low clinical mastitis incidence. *Small Rumin. Res.* 95, 193–196.
- Greek Payment and Control Agency for Guidance and Guarantee Community Aid, 2018. <http://www.opekepe.gr>, (Accessed 12 November 2018).
- Hellenic Statistical Authority, 2018. <http://statistics.gr>, (Accessed 12 November 2018).
- Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G., Jarvis, A., 2005. Very high resolution interpolated climate surfaces for global land areas. *Int. J. Climatol.* 25, 1965–1978.
- Kantzoura, V., Kouam, M.K., Teofanova, D., Theodoropoulos, G., 2011. Geographic distribution modelling for ruminant liver flukes (*Fasciola hepatica*) in south-eastern Europe. *Int. J. Parasitol.* 41, 747–753.
- Lacetera, N., Scalia, N., Bernabucci, U., Ronchi, B., Pirazzi, D., Nardone, A., 2005. Lymphocyte functions in overconditioned cows around parturition. *J. Dairy Sci.* 88, 2010–2016.
- Lecchi, C., Rota, N., Vitali, A., Cecilian, F., Lacetera, N., 2016. In vitro assessment of the effects of temperature on phagocytosis, reactive oxygen species production and apoptosis in bovine polymorphonuclear cells. *Vet. Immunol. Immunopathol.* 182, 89–94.
- Marco Melero, J.C., 1994. Mastitis in Laxta Breed Sheep: Epidemiology, Diagnosis and Control. Doctoral thesis. University of Zaragoza, Spain.
- Martin, J.G.P., Silva, G.D.E., da Fonseca, C.R., Morales, C.B., Silva, C.S.P., Miquelluti, D.L., Porto, E., 2016. Efficiency of a cleaning protocol for the removal of enterotoxigenic *Staphylococcus aureus* strains in dairy plants. *Int. J. Food Microbiol.* 238, 295–301.
- Neerinckx, S.B., Peterson, A.T., Deckers, J., Leirs, H., 2008. Geographic distribution and ecological niche of plague in sub-Saharan Africa. *Int. J. Health Geograph.* 7, 54.
- Pantoja, J.C.F., Reinemann, D.J., Rugg, P.L., 2011. Factors associated with coliform count in unpasteurized bulk milk. *J. Dairy Sci.* 94, 2680–2691.
- Peterson, A.T., Bauer, J.T., Mills, J.N., 2004. Ecologic and geographic distribution of feline coronavirus disease. *Emerg. Inf. Dis.* 10, 40–47.
- Phillips, S.J., Anderson, R.P., Schapire, R.E., 2006. Maximum entropy modeling of species geographic distributions. *Ecol. Model.* 190, 231–259.
- Raboisson, D., Derville, M., Herman, N., Cahuzac, S., Allaire, G., 2012. Herd-level and territorial-level factors influencing average herd somatic cell count in France in 2005 and 2006. *J. Dairy Res.* 79, 324–332.
- Rose, H., Wall, R., 2011. Modelling the impact of climate change on spatial patterns of disease risk: sheep blowfly strike by *Lucilia sericata* in Great Britain. *Int. J. Parasitol.* 41, 739–746.
- Simoës, G.H., Pozza, M.S.D., Zambom, M.A., Lange, M.J., Neumann, M.E., 2015. Dairy production system type and critical points of contamination. *Semina-Cienc. Agrar.* 36, 3923–3934.
- Valiakos, G., Giannakopoulos, A., Spanos, S.A., Korbou, F., Chatzopoulos, D.C., Mavrogianni, V.S., Spyrou, V., Fthenakis, G.C., Billinis, C., 2017. Use of geographical information system and ecological niche model to analyse potential exposure of small ruminants to *Coxiella burnetii* infection in central Greece. *Small Rumin. Res.* 147, 77–82.
- Vasileiou, N.G.C., Chatzopoulos, D.C., Gougoulis, D.A., Sarrou, S., Katsafadou, A.I., Spyrou, V., Mavrogianni, V.S., Petinaki, E., Fthenakis, G.C., 2018a. Slime-producing staphylococci as causal agents of subclinical mastitis in sheep. *Vet. Microbiol.* 224, 93–99.
- Vasileiou, N.G.C., Cripps, P.J., Ioannidi, K.S., Chatzopoulos, D.C., Gougoulis, D.A., Sarrou, S., Orfanou, D.C., Politis, A., Gonzalez, Calvo, Valerio, T., Argyros, S., Mavrogianni, V.S., Petinaki, E., Fthenakis, G.C., 2018b. Extensive countrywide field investigation of subclinical mastitis in sheep in Greece. *J. Dairy Sci.* 101, 7297–7310.
- Vasudevan, P., Nair, M.K.M., Annamalai, T., Venkitanarayanan, K.S., 2003. Phenotypic and genotypic characterization of bovine mastitis isolates of *Staphylococcus aureus* for biofilm formation. *Vet. Microbiol.* 92, 179–185.
- Williams, R.A., Peterson, A.T., 2009. Ecology and geography of avian influenza (HPAI H5N1) transmission in the Middle East and northeastern Africa. *Int. J. Health Geograph.* 8, 47.
- WorldClim – Global Climate Data, 2017. www.worldclim.org, (Accessed 30 November 2017).
- Zeimes, C.B., Olsson, G.E., Ahlm, C., Vanwambeke, S., 2012. Modelling zoonotic diseases in humans: comparison of methods for hantavirus in Sweden. *Int. J. Health Geogr.* 11, 39.