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Exposure of Dairy Cows to *Coxiella burnetii* in Greece: Surveillance Results and Association of Bacterial Presence with Environmental Variables

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Abstract: The exposure of dairy cows to *Coxiella burnetii* using molecular and serological techniques was investigated in this study. Bulk tank milk and serum samples were collected from various farms in Greece (mainly northern Greece). DNA extraction was performed on milk samples, and qPCR targeting the IS1111 insertion sequence was performed to detect bacterial pathogens. An ELISA was used to detect specific antibodies in bulk milk and individual serum samples. Data on farms were collected in the field using handheld Global Positioning System Garmin units. The collected data were analyzed using an Ecological Niche Model within the framework of a geographic information system. The results indicated that in more than half of the dairy farms (35/60, 58.3%), *C. burnetii* is present in milk. Specific antibodies were also detected in almost all milk samples (57/60, 95.0%). At least one seropositive animal was identified using ELISA in the majority of the examined farms (25/28, 89.3%). *C. burnetii* PCR-positive farms were located in the low-altitude zone with a mean value of 97 m above sea level (range: 2–681). The environmental variable with the highest gain when used in isolation is precipitation in the wettest quarter (28.3% contribution), which therefore appears to have the most useful information by itself. The environmental variable that decreases the gain the most when omitted is the minimal temperature of the coldest month (6.9% contribution). The analysis demonstrated that a mild climate with low precipitation favors a positive status. The exposure of dairy cattle farms to *C. burnetii* is massive, raising significant concerns regarding livestock production and public health implications.

Keywords: *Coxiella burnetii*; Q fever; cattle; Greece; exposure; ENM; GIS; maximum entropy

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1. Introduction

Q fever is a universally distributed zoonotic disease caused by *Coxiella burnetii*, an obligate intracellular Gram-negative bacterium of the family *Coxiellaceae* and order *Legionellales*. The microorganism was first isolated in 1935 in Australia, but it currently demonstrates worldwide dissemination, with the exception of New Zealand and Antarctica [1]. This distribution can be explained by its wide host range, low infective dose (only 1–10 organisms), and stability in variable environments for sufficient time periods [1]. Domestic ruminants

are considered the main reservoirs of the bacterium, and small ruminants (sheep and goats) have been systemically associated with human disease outbreaks [2].

The exposure of cattle to *C. burnetii* is undoubtedly high worldwide. However, infection in cattle is mostly asymptomatic, and the majority of animals are considered to be subclinical carriers of the bacterium [3]. When clinical signs occur, sporadic abortions and stillbirths may be observed, and *C. burnetii* has also been correlated with more non-specific reproductive disorders such as infertility, metritis, retained fetal membranes, and mastitis. The type of strain involved in infected livestock is a possible factor affecting clinical outcomes [4]. In recent years, the pathogen has been considered as the etiological agent of approximately 10% of abortions reported in cattle in various studies performed in different countries, e.g., Belgium, Italy, Brazil, and others [5–7]. A large study performed previously [8] showed that the main shedding routes in cattle are via the milk and vaginal mucus. In contrast, shedding of the pathogen is scarce and sporadic in cattle feces. This might be the reason why cattle have not been implicated in large human outbreaks of the disease in the past, contrary to small ruminants, where fecal shedding may be highly persistent. Nonetheless, it was recently proven that even in areas free from small ruminants, cattle may play a significant role in exposure to the pathogen, especially in professionals in direct contact with animals, such as veterinarians and farmers [9].

In humans, infection occurs in two main forms, acute and chronic Q fever. In the acute type, the patient is mostly asymptomatic (approximately 60% of the cases, where detection is based on seroconversion) or suffers from a mild, flu-like disease. In some cases, the clinical presentations may be more severe like atypical or rapidly progressive pneumonia, acute hepatitis, aseptic meningitis or encephalitis and acute endocarditis. In a limited number of infected patients (1–5%), the chronic type will eventually emerge. The more severe type is characterized by a focalized and persistent type of infection, mostly endocarditis, and non-specific symptoms, such as hepatosplenomegaly, fever, and weight loss [10]. Many concerns have arisen in recent years regarding underdiagnosed chronic cases, and in a recent study, it was estimated that approximately one-third of patients with culture-negative endocarditis and vascular infection were infected with *C. burnetii*. Hence, endocarditis and vascular infections may be significantly underestimated in routine clinical practice [11]. Moreover, Q fever during pregnancy has been correlated with abortion and prematurity, especially when acquired during the first or second trimester, respectively [10,12].

In Greece, *C. burnetii* infections are currently thought to be underdiagnosed due to physicians' lack of awareness [13]. Similarly, while there are a number of studies [14–17] investigating the distribution of *C. burnetii* in small ruminants, data on cattle are rather limited [18,19]. Members of our team previously investigated the exposure of small ruminants to *C. burnetii* by detecting antibodies against the pathogen in sheep and goat serum samples [16]. In this study, molecular and serological surveillance of large ruminant samples derived from farms in several regions throughout the country was initially performed. The collected data were geocoded and analyzed using an Ecological Niche Model (ENM), under the framework of a geographic information system (GIS). The specific objectives of the research were as follows: (i) to evaluate the distribution of *C. burnetii* exposure in cattle throughout Greece and (ii) to identify the environmental parameters associated with this exposure.

2. Materials and Methods

2.1. Study Area and Farm Selection

There are approximately 625.000 cattle and 11.000 farms in Greece [20]. The objective of this study was to provide sufficient data about the dissemination of *C. burnetii* in dairy cattle farms throughout Greece, and thus, sampling areas involved a variety of regions (Macedonia, Thrace, Epirus, Thessaly, Peloponnese, Central Greece, and South Aegean). Most of the selected farms were located in the northern regions, where most of the country's large ruminants are encountered (Figure 1).

Figure 1. Distribution map of sampled cattle farms in the country. The *Coxiella burnetii* PCR-positive farms are indicated.

The included farms were selected by satisfying additional requirements, such as breeding dairy cattle (Holstein breed), keeping an accessible, detailed file of recent clinical manifestations of any reproductive disorder, and collaboration potential with a local veterinarian. Herd size was variable (80–1000 animals), whereas the median number of animals in these farms was 200 per farm, and the mean number was 254 animals per farm.

2.2. Sample Collection

The samples were collected between March 2020 and January 2022. A sufficient quantity of bulk tank milk (approximately 200 mL) was aseptically collected from each of the selected farms in two sterile vials. On the same day, blood samples were obtained from cows with a recent history of reproductive disorders such as abortion, embryonic loss, infertility, metritis, and retained fetal membranes where available. An amount of 10 mL of blood was collected aseptically from the coccygeal vein of the cows and placed in red-top serum tubes. The blood remained in the tube before the centrifugation for 16 h on average and the transportation time was 12 h on average. No issues of hemolysis were detected

in the samples. The evaluation of the disorders was performed in collaboration with local veterinarians who had clinically diagnosed them.

The samples were maintained at 4 °C until they were transported to the laboratory. Milk samples measuring 50 ml were centrifuged at 2000× *g* for 20 min at 4 °C to separate the lactoserum from the cream. Cream was carefully discarded, and approximately 1.2 mL of lactoserum was collected in a 1.5 mL Eppendorf tube. A similar centrifugation process was applied to the blood samples. Extracted blood sera, lactosera, and the remaining quantity of bulk milk sample (almost 150 mL) was subsequently maintained at minus 20 °C, until further processing.

2.3. Serological Assays

Bulk tank milk and serum samples were examined for the presence of antibodies against *C. burnetii* using one of two commercial indirect ELISA kits (available in the two laboratories responsible for performing the ELISA tests), namely the IDEXX Q-Fever (*C. burnetii*) Antibody Test (IDEXX Laboratories, Inc.; Westbrook, ME, USA) and the ID Screen® Q Fever Indirect Multi species (ID-VET, Innovative Diagnostics; Grabels, France), which are coated with a mix of phase I and II antigens. All procedures were performed in accordance with the manufacturer's instructions and recommendations.

Briefly, for the IDEXX kit, serum samples were incubated at room temperature for 30 min and then diluted 1:400 using wash solution. The lactosera were examined after 1:5 dilution using wash dilution. Negative and positive controls were included when examining the samples. The ELISA results were obtained by comparing the optical density (OD) of the sample with that of the positive control. For each sample, we calculated the ELISA index S/P% according to the manufacturer's instructions using a photometer at a wavelength of 450 nm, as follows: S/P% = 100 × (OD value of the sample tested − OD value of the negative control)/(OD of the positive control − OD of the negative control). Serum and lactoserum samples with S/P% < 30% were considered negative, whereas those with S/P% ≥ 40 were considered positive. A sample with 30% ≤ SP% < 40% was considered suspected and re-analyzed.

For the ID-VET kit, the samples and reagents were incubated at room temperature for 30 min before use. Serum samples were tested at a final dilution of 1/50, whereas bulk milk samples were tested undiluted. Negative and positive controls were included in the assays at dilutions of 1/50. In addition, special washing precautions were taken when analyzing bulk milk samples. The results were obtained by calculating the optical density (OD) of each sample and the controls at a wavelength of 450 nm and using the value S/P% as follows: S/P% = 100 × (OD sample − OD negative control)/(OD positive control − OD negative control). Serum samples with S/P% < 40% were considered negative and those with S/P% ≥ 50 were considered positive, while samples with 40% ≤ SP% < 50% were considered suspect and examined again. In reference to bulk milk samples, S/P% < 30% was considered negative, and S/P% ≥ 40 was considered positive. If 30% ≤ SP% < 40%, the samples were considered suspected and reanalyzed.

Optical density of samples and controls was measured using MR-96A (Mindray, Shenzhen, China) or Multiskan FC microplate photometer (Thermo Fisher Scientific, Waltham, MA, USA).

2.4. Molecular Assays

Total DNA from the bulk tank milk samples was extracted using a commercial spin column kit (IndiSpin Pathogen Kit; INDICAL BIOSCIENCE GmbH, Leipzig, Germany). Briefly, a total of 1.5 mL of milk was transferred into a 2 mL Eppendorf tube and centrifuged for 20 min at 15,000× *g*. The supernatant was discarded, and the pellet was washed twice with 500 µL PBS. Finally, 400 µL of PBS buffer was added, the Eppendorf tube was vortexed at full speed for 1 min, and 200 µL was transferred to the spin column kit. The remainder of the procedure was performed in accordance with the manufacturer's instructions. qPCR was performed using a commercial kit (ADIAVET™ Coxiella Real-

Time, BioX Diagnostics, Rochefort, France) targeting the IS1111 sequence. Briefly, 20 µL of A5 solution was dispensed into each PCR tube. For each test, 5 µL of purified DNA or 5 µL of the control was added. The positive control included in the kit was prepared at 1/1000 dilution. qPCR was performed using the StepOnePlus Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA, USA). The following program was applied: 2 min at 50 °C, 10 min at 95 °C, 45 cycles for 15 s at 95 °C, and 1 min at 60 °C. The sample was considered positive if a characteristic amplification curve was observed in the FAM (Ct threshold approximately < 40). The kit detects in VIC and HEX the internal endogenous control GAPDH, normally found in the samples. DNA extraction and amplification for each sample are considered to be valid if at least a characteristic amplification curve is observed for *C. burnetii* (FAM) or for the internal control (VIC or HEX).

2.5. Geographical Information System

Data from each farm were collected in the field using a handheld global positioning system (GPS) Garmin unit. Subsequently, the respective geo-references were resolved at a specific farm level. ArcGIS Pro 3.1 GIS software (ESRI; Redlands, CA, USA) was used for description and further analysis of the collected information.

2.6. Environmental Parameters

Climatic factors were derived from WorldClim version 1.4. software [21]. GIS environmental layers were created for the analysis using ArcGIS 10.1 GIS software (ESRI, Redlands, CA, USA). All data layers were combined into a common projection, map extent, and resolution during MaxEnt modelling. Environmental parameters are within regions, with climate layers (climate grids) being a set of global with a spatial resolution of 1 km^2.

2.7. Ecological Niche Model

In numerous studies, data on species presence often failed to encompass the entire natural habitat range. Ecological Niche Modelling or species distribution modelling tools, such as MaxEnt [22], enable the depiction of the entire distribution range. MaxEnt employs a maximum entropy algorithm to calculate the species' realized niche and likelihood of occurrence. The model identifies locations with environmental conditions similar to those where a species has been observed in the past. The basic requirements in order to be able to identify this potential distribution include data on presence points (*C. burnetii* presence in livestock farms), as well as the environmental variables of the study zone. Initially, a niche is defined based on the environmental values that correspond to 'presence' data (in our case, livestock farms exposure to *C. burnetii*). The model then compares the environmental values of each raster cell in the study area with those of the species niche. Using this information, the model was able to estimate the probability of a species occurrence in each cell, and MaxEnt software version 3.3.3 was utilized in this study to forecast suitable environmental niches for *C. burnetii* exposure. In the MaxEnt modelling process, the study area pixels represent the area where the distribution of the MaxEnt probability is defined. Occurrence records in pixels serve as sample points, with environmental parameters (e.g., climate and topography) as features. The MaxEnt method requires only presence data, utilizing continuous as well as categorical data, and includes efficient deterministic algorithms and mathematical formulations [22]. *C. burnetii* PCR-positive farms in the area of Macedonia and Thrace were used as occurrence points in the ENM procedure, as these were the areas from which sufficient data (38/60, 63.3% of the total sampled farms) was collected and 26 from the 35 total PCR-positive farms were detected (Figure 2); most dairy farms in the country are located in this region. The accuracy of model predictions was evaluated using the mean area under the curve (AUC) of the receiver operating characteristic curve (ROC). To reduce the number of environmental variables that showed a significant influence on the model, the jackknife procedure was applied. Testing was repeated with the jackknife procedure until all the remaining variables had a positive effect on the total gain.

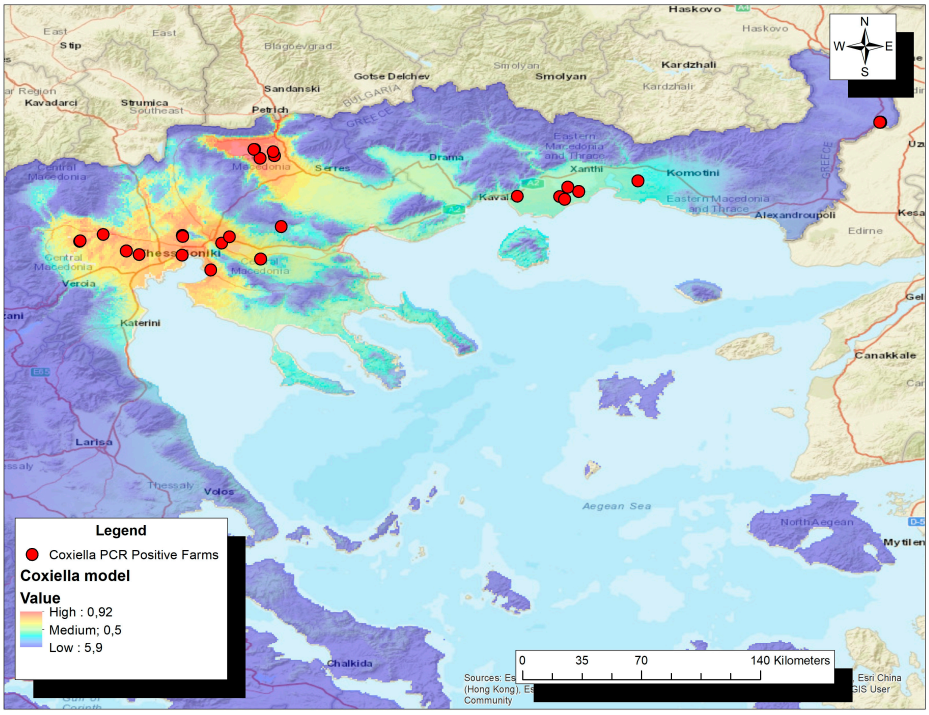

Figure 2. Distribution map of *Coxiella burnetii* PCR-positive farms used in the Ecological Niche Modeling. Colors represent probabilities of presence (from low/blue to high/red), choosing logistic output as it gives an estimate between 0 and 1.

3. Results

The laboratory results are summarized in Table 1. Detailed results are presented in the Supplementary file (Table S1). In 35 of the 60 bulk milk samples (58,3%) from the farms under investigation, *C. burnetii* was detected by qPCR. With reference to the ELISA results derived from the analysis of lactoserum samples, 57 samples (95%) were considered positive for the presence of specific antibodies (IgG). Unsurprisingly, all PCR-positive samples were found to be positive by ELISA.

Table 1. Molecular and serological surveillance results.

Farms	Herd Size	BTM PCR	BTM ELISA	Animal Serum ELISA
60	Mean = 250 (Range = 80–1000)	35/60 (58.3%, CI95% = 44.9–70.93%))	57/60 (95.0%, CI95% = 86.1–99.0%)	97/257 (37.7%, CI95% = 31.8–44.0%)

Farms located in Epirus and Thessaly demonstrated relatively lower rates of positivity when tested by PCR (1/7 and 1/4, respectively), whereas higher rates were detected in northern Greece (Western/Central Macedonia and Eastern Macedonia/Thrace, 19/28 and 7/10, respectively).

Regarding the results of serum samples, a total of 257 serum samples from animals with reproductive problems belonging to 28 farms were investigated: at least one seropositive animal per examined herd was identified by ELISA, in the majority of the examined farms (25/28, 89.3%). The majority of samples were collected from Central Macedonia (49/115, 42.6%) and Eastern Macedonia/Thrace (35/110, 31.8%), demonstrating high rates of seropositivity.

Positive farms were located at an altitude range of 2–681 m with a mean of 97 m, mainly in the lower altitude zone (<250 m above sea level).

The contribution of each environmental variable to the MaxEnt model analyzed in this study is presented in Table 2. Jackknife test results of variable importance for cattle exposure to *C. burnetii* in the areas of Macedonia and Thrace are shown in Figure 3.

Table 2. Contribution of environmental factors to the Maxent model used in this study.

Environmental Variable	Unit of Meas.	Code	% Contribution	Permutation Importance
Annual mean temperature	°C	bio1	0.1	0
Mean diurnal temperature range (Mean of monthly (max temp–min temp))	°C	bio2	4.4	3.8
Isothermality (BIO2/BIO7) (×100)	percent	bio3	0	0
Temperature Seasonality (standard deviation×100)	percent	bio4	4	0.1
Maximal temperature of the warmest month	°C	bio5	0	0
Minimal temperature of the coldest month	°C	bio6	6.9	64.1
Temperature annual range (BIO5-BIO6)	°C	bio7	1.5	0
Mean temperature of wettest quarter	°C	bio8	0	0
Mean temperature of driest quarter	°C	bio9	0.9	0
Mean temperature of warmest quarter	°C	bio10	0	0
Mean temperature of coldest quarter	°C	bio11	0.7	0.9
Annual precipitation	mm	bio12	1.3	0
Precipitation of the wettest month	mm	bio13	40.2	15.4
Precipitation of the driest month	mm	bio14	0	0
Precipitation seasonality (coefficient of variation)	percent	bio15	7,8	2.2
Precipitation of the wettest quarter	mm	bio16	28.3	0.3
Precipitation of the driest quarter	mm	bio17	0	0
Precipitation of the warmest quarter	mm	bio18	4	13.2
Precipitation of the coldest quarter	mm	bio19	0	0

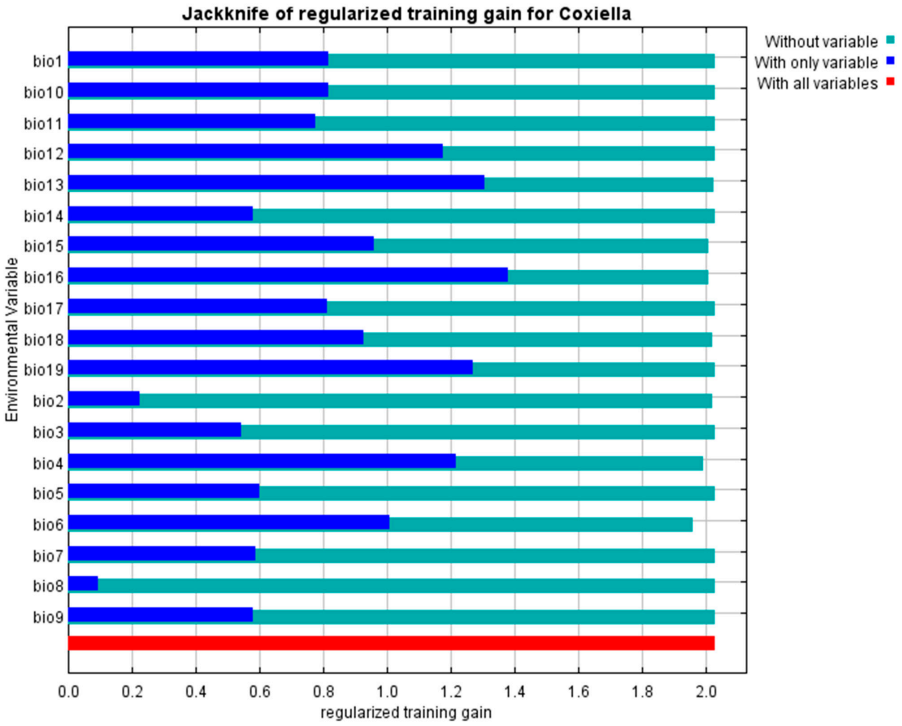

Figure 3. Jackknife test results for variable importance for cattle exposure to *C. burnetii* in the areas of Macedonia and Thrace. The Jackknife Figure shows the impact of each variable on the entire model and gives the function and signification of each variable. Blue color shows the independent contribution of this variable to the model and light blue gives the effect to the model if this variable is not included. The red bar represents the gain using all variables.

The environmental variable with the highest gain when used in isolation was bio16 (precipitation of the wettest quarter), which appeared to have the most useful information by itself. The response of *C. burnetii* to the bio16 variation is shown in Figure 4. The logarithmic probability of the presence of *C. burnetii* in cattle varied based on the precipitation of the wettest quarter. In particular, as the precipitation increased, the possibility of the presence of *C. burnetii* decreased.

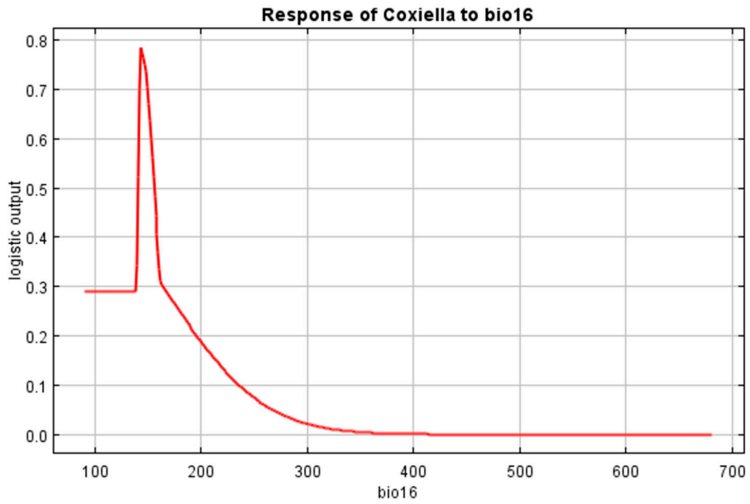

Figure 4. The response curve derived from the MaxEnt model showed the influence of the predictor variable bio16 (precipitation of the wettest quarter) on the probability of occurrence of *C. burnetii* in cattle in the areas of Macedonia and Thrace. In other words, the curve shows how *C. burnetii* predicted probability of presence changes as the variable varied. The unit in *x*-axis is in mm of precipitation.

The environmental variable that decreased the gain the most when it was omitted was bio6 (minimal temperature of the coldest month), which therefore appeared to have the most information that was not present in the other variables, with a minimum temperature of the coldest month above 0 °C being associated with the presence of the bacterium (Figure 5).

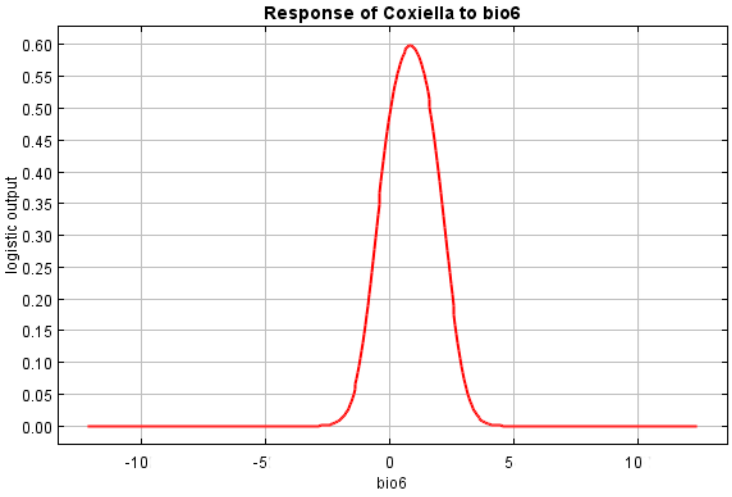

Figure 5. Response curve derived from the MaxEnt model showing the influence of the predictor variable: bio6 (minimal temperature of the coldest month) on the probability of occurrence of *C. burnetii* in cattle in the areas of Macedonia and Thrace. In other words, the curve shows how *C. burnetii* predicted probability of presence changes as the variable varied. The unit in the *x*-axis is in degrees of Celsius.

4. Discussion

To our knowledge, this study is the first to analyze the correlation between cattle exposure to *C. burnetii* in Greece and specific environmental factors.

The aforementioned results, especially those from bulk milk samples, indicate that *C. burnetii* is widely distributed in bovine farms throughout the country, in accordance with the results of a previous countrywide study [19]. Similar data are available for other European countries too [23,24].

Higher seropositivity rates were detected in individual animal samples than in a previous study [18]. Animals with a history of reproductive disorders were included in both studies. However, the number of tested animals was significantly higher in this study, and the origin of the samples was dissimilar; thus, this variation could not be evaluated. In addition, molecular testing of bulk tank milk samples used in this study in parallel with serological test allowed the demonstration of the current circulation of the bacterium in the time of sampling.

In addition, seroconversion should be evaluated with caution since it can be affected by several factors, especially in endemic herds. Seropositive animals do not always shed *C. burnetii* in their milk, and shedding of the pathogen in milk is intermittent; however, it has been established that persistent shedder cows are mostly highly seropositive [8,25]. In a relevant study, the seroconversion rate was found to be significantly higher in primiparous cows than in multiparous cows, whereas several multiparous cows remained persistently seronegative [26]. Moreover, the presence of concurrent pathogens can also influence seropositivity. For example, since animals infected with *Neospora caninum* produced reduced titers of antibodies against *C. burnetii*, the possibility of cross-protection was suggested in animals concurrently infected with both agents [27]. Thus, several factors must be co-estimated during the evaluation of individual serological results. Therefore, additional diagnostic strategies are crucial for determining the infectivity status of a herd.

In reference to the geographic distribution of infected herds, high rates documented in northern Greece are also in accordance with previous surveillance research [19]. Central Macedonia has the greatest bovine population in the country [20]; therefore, the presence of the pathogen in local farms is a matter of concern.

Moreover, the greater danger of animal exposure to *C. burnetii* in farms located in plain terrains has already been documented for small ruminants in the region of Thessaly [16]. In addition, open landscapes and climatic factors such as high winds, mild temperatures, and relatively low precipitation, which are common in these areas, have been associated with an increased risk of dissemination [28,29]. An additional risk factor is the presence of ticks, and the conditions for ticks are usually preferable in these regions. As the altitude decreases, the risk of exposure to infected ticks increases. The area under study is located at a relatively low altitude. Thus, we expect that ticks are widely distributed and may be frequently related to the transmission of various pathogens to humans and animals, including *C. burnetii* [30].

Based on the models used in this study, the precipitation of the wettest quarter, followed by the precipitation of the wettest month, and the minimal temperature of the coldest month were the variables with the largest contribution to the model; areas with relatively low precipitation and high minimal temperature demonstrate a high presence of the pathogen. This is in agreement with the ecological and biological features of *C. burnetii.* This obligate intracellular pathogen produces endospore-like forms, which can survive for long periods in the environment, and a hot and dry environment (high temperature and low precipitation) favors the wind-borne spread of infected aerosols [31]. It has recently been shown that high wind speed and high bacterial load in contaminated aerosols play a significant role in the transmission of the pathogen to an infection-free herd [32].

Finally, in a recent study in Greece, the genotype of *C. burnetii* isolated from dairy cattle was related to genotypes of ovine or caprine origin, indicating the danger of transmission between herds in the vicinity of small ruminants [19]. On the other hand, good hygiene

practices, such as frequent bedding cleaning, lower density, and automatic milking systems were identified as protective factors [28].

Since the presence of *C. burnetii* in a farm has been established, the herd acts as a reservoir, enhancing its distribution. Infected cows spread the bacterium through vaginal mucus, feces, and milk, even when they are asymptomatic and parturition is not affected. This shedding sometimes persists for several months [33]. Therefore, cattle herds are potentially significant bacterial reservoirs, and the danger of transmission exists, as has been identified through antibody production and *C. burnetii* detection, even in asymptomatic herds [33].

In the present study, a total of 60 farms were analyzed, but only the molecular positive herd samples from the areas of Macedonia and Thrace (Northern Greece) (n = 26) were used for MaxEnt modelling because the Maxent program develops models of species' distributions using species' presence data and environmental data [22,34,35]. However, Papeş and Gaubert [36] reported that approximately 15 records represents the minimum requirement for achieving reliable modelling results using Maxent. Moreover, based on Pearson et al. [37], it is possible to obtain some statistical significance with only five records when modelling using Maxent. Nonetheless, a larger and more dispersed sampling effort is required to determine a more accurate (based on models) distribution of the presence of *C. burnetii* in cattle throughout Greece, even though the majority of cattle farms are located in the main sampling area of this study. Moreover, in order to estimate the impact of the pathogen on livestock production, it is important to organize active surveillance strategies that will focus on the molecular identification of the pathogen on samples of the reproductive system, including vaginal swabs, placenta swabs focusing on the zones of necrosis, cotyledons presenting lesions, organs (spleen, lung, liver), or the stomach contents of the aborted fetus, are the most suitable samples for evaluating the impact of the disease on cattle farms with regard to reproductive problems [38].

The study has certain limitations: Most samples are collected in specific areas where the majority of cattle farms are located in Greece. This reduces the variance of the analyzed environmental variables. Demonstrating the presence of the bacterium does not prove the causality of the pathogen for the reproduction problems occurred in the farms. Future studies must also quantify the presence of the bacterium in samples derived from animals with reproductive disorders, as indicated by the relevant EFSA report [38]. Finally, it is important to combine data from various sources, e.g., small ruminants, humans, and the environment, in order to accurately estimate the impact of the bacterium presence in all its aspects, following a One Health perspective.

The present work can be considered the first step towards a more accurate approximation of the distribution of this pathogen of significant importance in the country and towards the identification of all environmental variables that affect it. Our study provides information that might be essential when designing eradication strategies for *C. burnetii* in countries with variable geographical landscapes, such as Greece.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/microbiolres15020043/s1, Table S1: Results of molecular and serological investigation for the presence of *Coxiella burnetii* per cattle farm.

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