Original Article

Multizonal Analysis of Bioclimatic and Topographic Factors on Modeling of *Dicrocoelium dendriticum* in Iran

Abbas Oliya Ardekani[1], Behnam Meshgi[1*], Ahmad Ali Hanafi-Bojd[2,3], Seyed Hossein Hosseini[1], Shirin Khodabakhsh Arbat[1]

1. Department of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
2. Department of Vector Biology and Control of Diseases, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.
3. Zoonoses Research Center, Tehran University of Medical Sciences, Tehran, Iran.

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ABSTRACT

Background: Dicrocoeliasis, caused by *Dicrocoelium dendriticum*, is a significant parasitic disease with a complex life cycle that affects livestock worldwide, particularly in temperate and Mediterranean regions.

Objectives: This study investigated the relationship between topographic and bioclimatic variables and the parasite's distribution to identify key factors influencing disease occurrence.

Methods: During the fall of 2022, 1,030 fecal samples were collected from four climatic zones in Iran: cold and semi-arid, cool and sub-humid, warm and hyper-arid, and mild and humid. The samples were analyzed using the McMaster flotation technique, and the results were further examined with ArcGIS and MaxEnt modeling to assess spatial and environmental patterns.

Results: The highest infection rate (52.31%) was observed in the cold and semi-arid climate. While the lowest (30.09%) was recorded in the warm and hyper-arid climate. Jackknife analysis identified the normalized difference vegetation index (NDVI) as the most significant topographic factor influencing parasite distribution in three of the four climatic zones. The mean temperature of the coldest quarter (Bio 11) was the most influential bioclimatic variable. Slope emerged as the most critical topographic factor in the mild and humid climate. At the same time, the precipitation of the driest quarter (Bio 17) and the precipitation of the driest month (Bio 14) were the most impactful bioclimatic variables. These patterns show how topographic and bioclimatic factors affect the distribution of *D. dendriticum* in various climate zones.

Conclusion: This study also provides helpful information for developing improved control strategies and enhancing livestock management in affected areas, thereby leading to more effective control of this parasite.

Keywords: Bioclimate, *Dicrocoelium dendriticum*, Modeling, Spatial distribution, Topography

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*** Corresponding Author:**
Behnam Meshgi, Professor.
Address: *Department of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.*
Phone: *+98 (21) 66929532*
E-mail: *bmeshgi@ut.ac.ir*

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Introduction

With the growing global population, the demand for nutritious and healthy meat continues to rise. In Iran, meat consumption predominantly relies on ruminant sources, such as sheep and goats. This preference is influenced by factors including local vegetation, climatic conditions, dietary habits, and religious beliefs (Mazinani & Rude, 2020; Kamalzadeh et al., 2008). According to the latest data from the Statistical Center of Iran, the sheep and goat population is approximately 70 million, the highest among livestock species. These animals are primarily raised through free grazing systems (Statistical Center of Iran, 2024).

As public access to food safety information increases, societal trends have shifted toward a preference for high-quality, health-conscious meat products. This shift is driven by greater awareness of the nutritional value and safety standards associated with meat consumption (Piggott & Marsh, 2004). Consequently, consumers are increasingly prioritizing meat that is safe, nutritious, and produced under stringent health regulations. This trend has significant implications for the meat industry, compelling producers to enhance quality standards and ensure product safety to meet evolving public health demands (Teixeira & Rodrigues, 2021). However, the presence of pathogenic factors in the environment, particularly parasites, poses a significant challenge, increasing the risk of livestock contamination and adversely affecting food safety and the broader food industry (Gabriël et al., 2022; Teixeira & Rodrigues, 2021).

Among parasitic pathogens, *D. dendriticum* (commonly known as the "lancet fluke") is of particular concern for livestock worldwide, especially in temperate and Mediterranean regions. This trematode primarily resides in the bile ducts and gallbladder of domestic and wild ruminants, causing significant economic losses through reduced productivity, liver condemnation during slaughter, and costly treatment measures (Meshgi et al., 2012; Mohammed & Alobaidii, 2024). Infected animals may exhibit clinical symptoms, such as decreased growth, reduced feed intake, diminished milk production, anemia, and photosensitization (Khan et al., 2021). Although *D. dendriticum* infections are treatable, they impose economic burdens on the livestock industry, particularly due to the rejection of livers affected by cholangitis during meat inspections (Chougar et al., 2019).

Historical parasitological studies have suggested that *D. dendriticum* may have zoonotic potential, often presenting without clear symptoms. For example, Bouchet and Bentrad identified *D. dendriticum* in latrine remains dating from the 11th to 16th centuries in France. Similarly, the parasite was detected in samples from the United Kingdom approximately nine centuries ago (Bouchet & Bentrad, 1997). More recent findings include evidence of *D. dendriticum* in a site in Kiasar, Iran, dating back to 250 BCE (Bizhani et al., 2017).

The life cycle of *D. dendriticum* is notably complex, requiring at least six months under favorable environmental conditions. Infection in definitive hosts, primarily ruminants, occurs during the grazing season when animals ingest ants carrying infective metacercariae (Jeandron et al., 2011; Van Paridon et al., 2017). The life cycle involves terrestrial mollusks as the first intermediate host, which excrete slime balls containing metacercariae. These slime balls are subsequently consumed by ants, the second intermediate host (Gürelli, 2017).

Although several studies have investigated the epidemiology of *D. dendriticum*, limited research has focused on the geographical and climatic factors influencing its prevalence (Díaz et al., 2007; Meshgi et al., 2019; Otranto & Traversa, 2003). Factors, such as precipitation during the driest quarter, altitude, temperature seasonality, and precipitation during the driest month were found to be the most significant factors affecting the presence of *D. dendriticum* in Northern areas of Iran (Meshgi et al., 2019).

The present study aimed to conduct a spatial analysis of *Dicrocoelium* sp. in sheep flocks across four distinct climatic regions of Iran. Specifically, the study sought to (1) analyze the spatial patterns of *Dicrocoelium* infections in sheep and (2) investigate the key climatic variables influencing the parasite's distribution. By identifying the geographical and climatic factors associated with dicrocoeliasis, this research aimed to provide valuable insights to inform prevention and control strategies, ultimately mitigating the impact of this parasitic disease on animal and public health.

Materials and Methods

Study area and sample collection

This study was conducted over a six-month period in the fall and winter of 2022. A total of 1,030 fecal samples were collected from four provinces in Iran, each characterized by distinct climatic conditions: West Azerbaijan Province (cold and semi-arid; n=151), Gilan Province (mild and humid; n=354), Kurdistan Province (cool and sub-humid; n=196), and Semnan Province (warm and hyper-arid; n=329). Six cities were selected from each climate zone, and 4-12 villages were sampled from each city (Table 1).

West Azerbaijan, covering an area of 29,137 km², is the 13th largest province in Iran, representing 2.25% of the country's total land area (coordinates: 37°33′10.08″ N, 45°4′33.24″ E). Located in the northwest, the province features a large, mountainous region with diverse topography. Its borders with Turkey and Iraq are marked by high, snow-covered mountains that stretch from north to south, with numerous rivers formed by snowmelt flowing through the valleys. The Hercynian forests along the Caspian Sea form a green belt approximately 110 km wide and 800 km long, dominated by tree species, such as maple. This ecoregion receives between 600 and 2,000 mm of rainfall annually. Gilan, situated on the western edge of the Alborz mountain range and covering an area of 14,042 km² (coordinates: 37°26′ N, 49°33′ E), is characterized by its plains along the Caspian Sea and the Talesh highlands to the northwest, which serve as a natural barrier separating it from East Azerbaijan. The region receives abundant rainfall and a mild climate, making it suitable for agriculture and livestock. Kurdistan, with an area of 29,137 km² (coordinates: 35°18′40.68″ N, 46°59′45.6″ E), accounts for approximately 1.7% of Iran's total landmass. Its mountainous terrain, influenced by its climate, is home to vast, green landscapes, ideal for sheep and goat farming. Semnan, spanning 96,816 km² (coordinates: 35°34′36.84″ N, 53°23′43.08″ E), features a diverse range of vegetation and climate zones. The northern regions, such as Shahmirzad and Mehdishahr, exhibit climate characteristics similar to those along the Caspian Sea, while the eastern area around Shahrud experiences a moderate, forested climate. The central, western, and southern areas, including the city of Semnan, are characterized by hot, dry conditions.

Fecal sample collection

The sampling procedure began with the random collection of fecal samples from free-grazing native sheep. Samples were collected from three separate flocks at each location, with 3 to 5 samples per flock, depending on flock size. The samples were individually packaged, labeled, and transported to the laboratory for analysis.

To examine the samples, the flotation method (McMaster technique) was employed using saturated NaCl and $ZnCl_2$ solutions with specific gravity of 1.53 (Ballweber et al., 2014; Majidi-Rad et al., 2018). This technique allowed for the quantification of *D. dendriticum* eggs per gram of feces (EPG). The results were recorded and formatted into a CSV file using Excel software.

Bioclimatic and topographic variables

For climate zones, 19 bioclimatic variables (referred to as "BIO"), such as temperature seasonality (Bio 4), and four topographic variables (including aspect, altitude, slope, and normalized difference vegetation index [NDVI]), were obtained at a spatial resolution of 1 km² from the WorldClim global climate databas. These variables were derived from continuous studies of monthly average temperature and precipitation data collected over the last 50 years of the 20th century (1950–2000) (Table 2).

Using ArcGIS 10.8 spatial analysis tools, probability maps indicating the presence of *D. dendriticum* were generated. Since the bioclimatic and topographic data are global in scope, the SDM toolbox in ArcGIS was utilized to clip all layers to the boundaries of Iran. Additionally, the variables were converted from TIF format to ASCII raster format in ArcMap 10.8 for application into the MaxEnt model.

MaxEnt modelling

All bioclimatic and topographic variables, along with regional data points on *D. dendriticum* contamination, were input into MaxEnt (version 3.4.4) using the aforementioned formats. The output was a spatial distribution map of dicrocoeliasis. The model was then employed to assess the effects of various topographic and bioclimatic factors on the parasite's distribution. In the MaxEnt model, 80% of the regional data points were randomly selected for training, while the remaining 20% were used to evaluate the model's predictive performance.

The model's performance was assessed using the receiver operating characteristic (ROC) curve and the area under the curve (AUC) metric. This analysis incorporates both sensitivity and 1-specificity. Sensitivity is a critical metric for evaluating diagnostic tests, representing the test's ability to correctly identify true positive cases. It indicates the percentage of actual positive cases that are accurately detected. Specificity, or the true negative rate, measures the proportion of negative cases that are correctly identified as negative. AUC values range from 0.5 to 1.0, with values above 0.9 indicating strong predictive power for distinguishing between positive and negative cases.

The contributions of individual variables and their combined effects were analyzed through a jackknife analysis, which evaluates the gain associated with each variable. Additionally, response curves were generated to illustrate the influence of each variable on the model's output (Lobo et al., 2008).

Table 1. Frequency and intensity of *D. dendriticum* infection of sheep across the four climatic zones

Locality		*D. dendriticum* Infection in Sheep	
Climate, Province	**City**	**No. (%)**	**Severity (EPG)**
Cold and semi-arid, West Azerbaijan (n=151)	Maku	36(52.77)	22.36
	Naqadeh	35(54.28)	38.25
	Khoy	23(65.2)	35.26
	Miandoab	17(47.05)	15.05
	Oshnavieh	22(54.54)	38.13
	Sardasht	18(33.33)	23.72
Totally frequency		52.31	29.64
Warm and hyper-arid, Semnan (n=329)	Semnan	58(27.58)	10.12
	Damghan	47(17.02)	19.33
	Shahrud	60(36.66)	37.27
	Garmsar	49(10.2)	2.77
	Sorkheh	54(29.62)	18.62
	Mehdishahr	61(52.45)	3.48
Totally frequency		30.09	16.19
Cool and sub-humid , Kurdistan (n=151)	Marivan	34(41.17)	15.82
	Sanandaj	33(45.45)	31.12
	Bijar	30(46.66)	28.83
	Saqqez	36(33.33)	20.02
	Kamyaran	34(47.05)	39.44
	Ghorveh	29(48.27)	32.51
Totally frequency		43.14	27.72
Mild and humid, Gilan (n=354)	Rudbar	66(42.42)	23.36
	Shaft	65(26.15)	13.77
	Siahkal	55(41.81)	14.77
	Masal	57(35.08)	30.5
	Talesh	58(43.1)	26.02
	Rudsar	53(28.3)	20.07
Totally frequency		36.15	21.36

Note: Sampling was conducted at 300 local points.

Table 2. Bioclimate and topographic factors in the present study

Variable Category	Environmental Variables	Code
Bioclimate	Annual mean temperature (˚C)	Bio 1
	Annual mean diurnal range (˚C)	Bio 2
	Isothermality (˚C)	Bio 3
	Temperature seasonality (standard deviation) (˚C)	Bio 4
	Maximum temperature of the warmest month (˚C)	Bio 5
	Minimum temperature of the coldest month (˚C)	Bio 6
	Annual temperature range (˚C)	Bio 7
	Mean temperature of the wettest quarter (˚C)	Bio 8
	Mean temperature of the driest quarter (˚C)	Bio 9
	Mean temperature of the warmest quarter (˚C)	Bio 10
	Mean temperature of the coldest quarter (˚C)	Bio 11
	Annual precipitation (mm)	Bio 12
	Precipitation of the wettest month (mm)	Bio 13
	Precipitation of the driest month (mm)	Bio 14
	Precipitation seasonality (coefficient of variation) (%)	Bio 15
	Precipitation of the wettest quarter (mm)	Bio 16
	Precipitation of the driest quarter (mm)	Bio 17
	Precipitation of the warmest quarter (mm)	Bio 18
	Precipitation of the coldest quarter (mm)	Bio 19
Topographic	Elevation(m)	Altitude
	Slope aspect (°)	Aspect
	Slope (°)	Slope
	Normalized difference vegetation index	NDVI

Results

The findings of this study are presented in two main sections. The first section examines the frequency of *D. dendriticum* infection in sheep across four distinct climatic zones. The second section analyzes the spatial distribution of the infection and identifies key risk factors using geographic information system (GIS) tools. These aspects are elaborated upon in the subsequent subsections.

Frequency and intensity of *Dicrocoelium* infection

The highest frequency of infection was observed in the cold and semi-arid climate of West Azerbaijan Province (52.31%), while the lowest was recorded in the warm and hyper-arid climate of Semnan Province (30.09%). Among the surveyed cities, Khoy (65.20%), Oshnavieh (54.54%), and Naqadeh (54.28%) exhibited the highest frequency of *Dicrocoelium*, whereas Garmsar (10.20%) and Damghan (17.02%) had the lowest contamination levels.

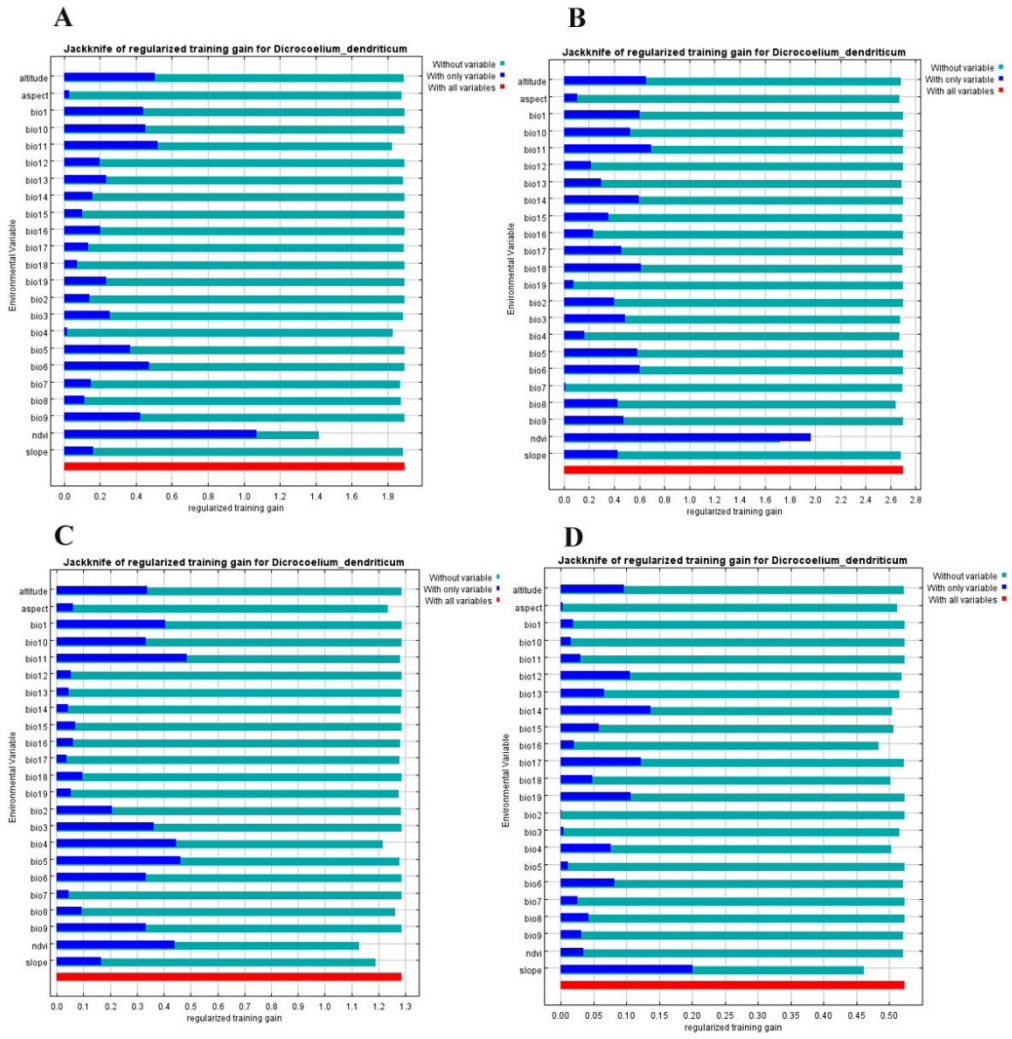


Figure 1. Jackknife analysis of the relative importance of different variables

A) Cold and semi-arid, B) Warm and hyper-arid, C) Cool and sub-humid, D) Mild and humid

Note: The red bar represents the total gain of all variables in the MaxEnt model. The light blue bar depicts the gain when a given variable is omitted; a lower gain indicates that the variable contains information that is not present in the other variables. The dark blue bar indicates the gain obtained using only the specific variable (i.e. its contribution).

Across the sampled villages, 22.2%, 16.6%, and 5.6% of those in West Azerbaijan, Kurdistan, and Semnan provinces, respectively, demonstrated 100% infection rates. Conversely, infection was absent in 17.1%, 27.7%, 28.5%, and 52.8% of the villages in Gilan, West Azerbaijan, Kurdistan, and Semnan provinces, respectively. The severity of infection, measured as EPG, ranged from 21 to 84 across all studied cities. The most heavily contaminated sample originated from Metesh village in Talesh, with an EPG of 131 (Table 2).

Contribution of variables and predicted distribution by MaxEnt

The importance of bioclimatic and topographic factors was evaluated using the jackknife method within each climatic zone. Jackknife analysis revealed that the NDVI was the most influential factor in determining the distribution of *Dicrocoelium* across three of the four climates (Figure 1). However, in mild and humid climates, slope was the most significant factor influencing the distribution of *Dicrocoelium*.

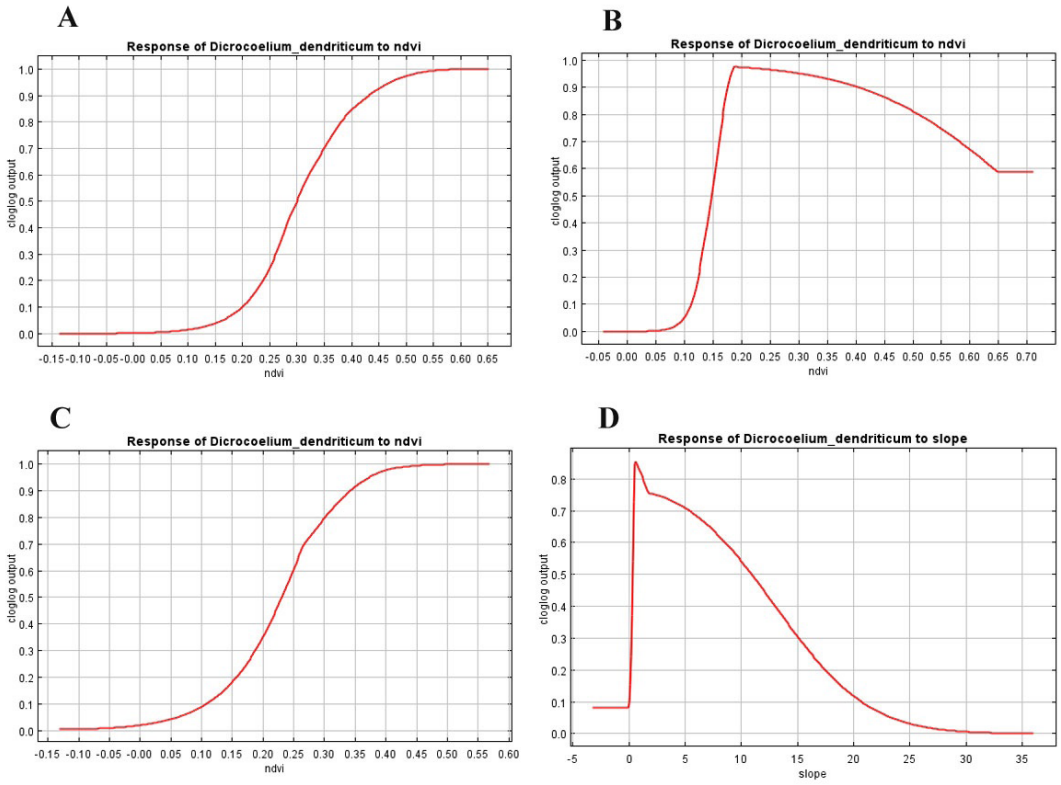


Figure 2. Response curves for suitable variables in the logistic-output format across climatic zones

A) Cold and semi-arid, B) Warm and hyper-arid, C) Cool and sub-humid, D) Mild and humid

The analysis highlighted the contributions of the variables to the MaxEnt model using three bars. The red bar represents the total gain of all variables in the MaxEnt model. The light-blue bar shows the gain when a specific variable is omitted; a lower gain indicates that the omitted variable contains critical information that is not provided by the other variables. The dark-blue bar represents the gain when only the specific variable is included, indicating its contribution to the model. The relative importance of the variables is summarized in Figure 1.

Importance of topographic and bioclimatic variables

In the cold and semi-arid climate, parasite presence was zero until vegetation-cover density reached 0.7, beyond which its probability increased significantly and remained constant (Figure 2A). The mean temperature of the coldest quarter (Bio 11) was the second most influential variable. In the warm and hyper-arid climate, parasite presence was absent up to an NDVI value of 0.05. From 0.05 to 0.2, the probability of infection increased sharply to nearly 100%. Beyond 0.2, the probability gradually decreased until stabilizing at approximately 0.6 (Figure 2B). Bio 11 also played a critical role in this climate. In the cool and sub-humid climate, the probability of parasite presence was zero up to an NDVI value of -0.07. Between -0.07 and 0.45, the probability increased gradually, reaching a peak of 100% (Figure 2C). In this climate, Bio 11 was also the second most significant factor. In the mild and humid climate, parasite presence increased with slope up to 1% but declined sharply beyond that point, reaching zero at a slope of 30% (Figure 2D). Precipitation during the driest quarter (Bio 17) and the driest month (Bio 14) were the most influential bioclimatic variables in this region. Response curves illustrating the relationships between the key variables and parasite distribution in each climate are shown in Figure 2.

Spatial distribution patterns

The MaxEnt model generated spatial distribution maps revealing distinct habitat suitability patterns across the studied regions. The cold and semi-arid region (West Azerbaijan Province) exhibited four major infection hotspots, two of which were concentrated near Lake Urmia.

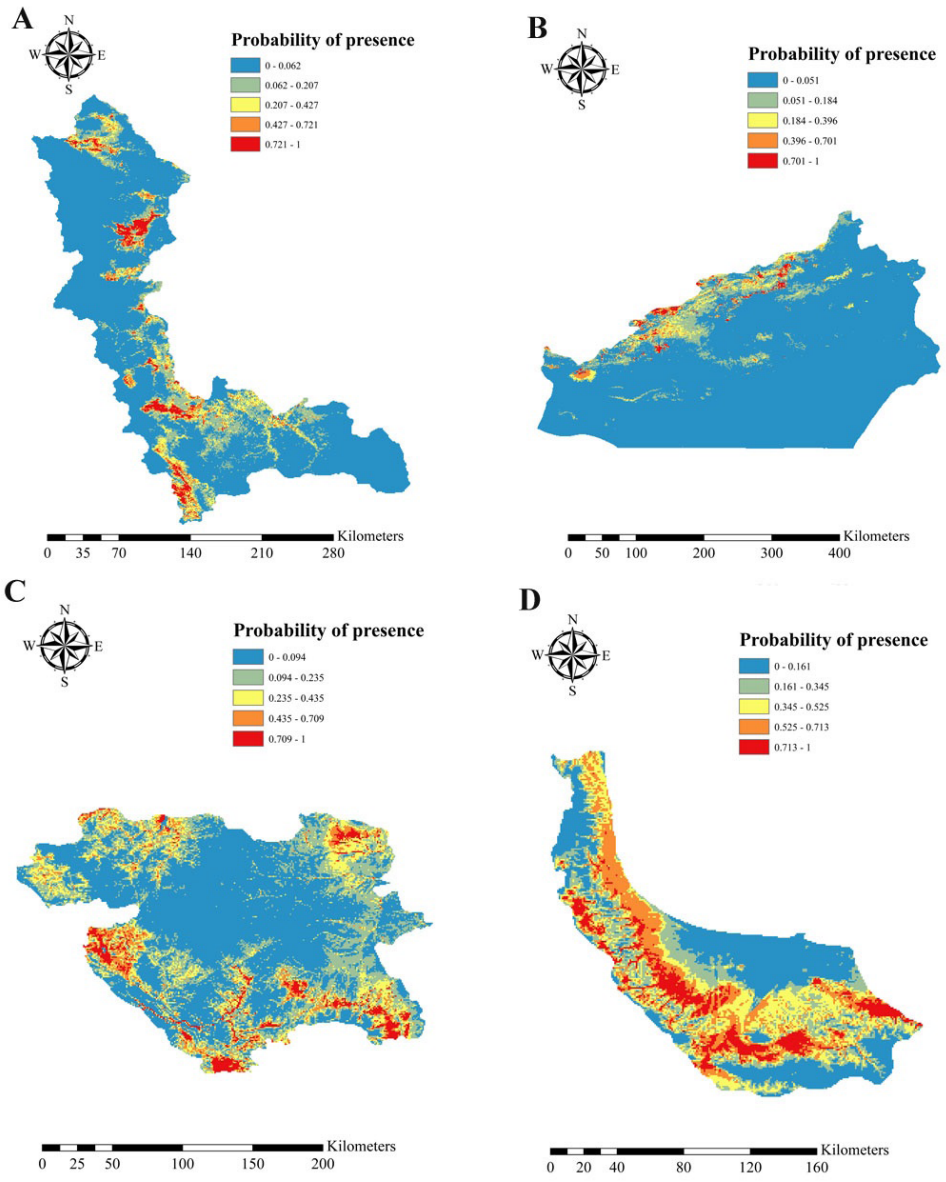


Figure 3. Spatial distribution patterns of Dicrocoelium generated by MaxEnt for each climatic zone

A) Cold and semi-arid, B) Warm and hyper-arid, C) Cool and sub-humid, D) Mild and humid

The highest suitability in the warm and hyper-arid region of Semnan Province was observed in the northern areas bordering Mazandaran Province. The cool and sub-humid region of Kurdistan Province showed elevated suitability along its southeastern to southwestern borders, whereas the central regions of Gilan Province, which has a mild and humid climate, showed the highest suitability. These spatial patterns highlight the complex interplay between topographic and bioclimatic factors in shaping *Dicrocoelium* distribution across different climatic zones (Figure 3).

Discussion

This study offers a comprehensive analysis of the distribution patterns and risk factors associated with *D. dendriticum* infection across four distinct climatic zones in Iran. The findings demonstrate significant spatial heterogeneity in parasite prevalence, emphasizing the complex interplay between topographic and bioclimatic variables in shaping the parasite's distribution. The prevalence of *D. dendriticum* varied notably across climatic regions, with the highest contamination rate (52.31%) observed in the cold and semi-arid climate of West Azarbaijan Province. This pattern indicates that the parasite thrives

in environments characterized by cold winters and mild summers, aligning with previous research conducted in the cold and semi-arid climate of the Kandi region (Godara et al., 2014).

Consistent with the findings of this study, Meshgi et al. (2019) reported higher infection rates of *D. dendriticum* in the northwestern provinces of Iran. They attributed this pattern to factors, such as soil type, the abundance of land snails and ants, and the presence of mountainous regions—environmental conditions that likely enhance the parasite's life cycle and transmission. Similarly, Díaz et al. (2007), in a comprehensive study involving 1,148 beef cattle across 170 farms in northwest Spain, identified mountainous areas as high-risk zones for *Dicrocoelium* infection. Key environmental factors included slopes steeper than 25%, elevations above 600 meters, annual rainfall exceeding 1,000 mm, and average temperatures below 11 ˚C.

These findings align with our observations, particularly in Gilan Province, where slope was identified as a significant factor influencing the distribution of the parasite. Furthermore, our results corroborate previous studies linking altitude to parasite prevalence. For example, Otranto and Traversa (2002) demonstrated that higher altitudes create optimal conditions for the development of *Dicrocoelium* intermediate hosts. This association may be explained by the increased humidity in highland areas, which supports favorable conditions for the parasite's life cycle and transmission. As demonstrated in previous studies, various factors, including climatic conditions, geographical parameters, diversity of intermediate hosts, and the persistence of parasite eggs in the environment, collectively influence the prevalence and distribution of dicrocoeliasis (Barve et al., 2011; Khan et al., 2023; Quiner & Nakazawa, 2017). This multifactorial nature of parasite distribution highlights the importance of employing advanced modeling approaches, such as the MaxEnt analysis utilized in this study.

The jackknife analysis identified the NDVI as the most influential factor in the distribution of *Dicrocoelium* across three of the four provinces examined. This underscores the critical role of vegetation cover in providing suitable habitats for intermediate snail hosts and facilitating parasite transmission. In Gilan Province, slope emerged as the most significant topographic variable influencing the parasite's distribution, suggesting that topography plays a vital role in shaping the spatial patterns of *D. dendriticum*.

These findings align with previous studies, which have demonstrated the utility of GIS-based techniques in elucidating the spatial distribution of *D. dendriticum* and identifying key environmental drivers (Khan et al., 2023; Meshgi et al., 2019). Such approaches offer valuable insights for designing targeted interventions and improving the management of parasitic infections.

Moderate vegetation density and gentle slopes provide optimal habitats for intermediate hosts, thereby promoting the transmission of *Dicrocoelium*. Conversely, dense vegetation or steep terrain may impede the movement and dispersal of both definitive and intermediate hosts, potentially restricting the parasite's spread. These intricate interactions between topographic features and parasite distribution underscore the complex ecological dynamics that shape the spatial patterns of dicrocoeliasis. Habitat suitability maps generated using MaxEnt revealed distinct spatial patterns across the study regions. Notably, areas surrounding Lake Urmia, situated in the cold, semi-arid climate of West Azerbaijan Province, emerged as significant infection hotspots. The interplay of moderate vegetation cover and gentle topography in this region likely creates ideal conditions for the survival and proliferation of intermediate hosts, thereby enhancing the parasite's transmission potential.

In the warm and hyper-arid climate of Semnan Province, the highest habitat suitability for *D. dendriticum* was observed in the northern regions bordering Mazandaran. Although vegetation cover in this area is relatively sparse compared to other zones, a specific combination of factors, including temperature, precipitation, and topography of the region appears to create suitable conditions for the parasite's life cycle and transmission. Proximity to Mazandaran, which likely has more favorable topographic and climatic characteristics, may also contribute to the elevated habitat suitability in northern Semnan. In the cool and sub-humid climate of Kurdistan Province, the southeastern to southwestern borders exhibited high habitat suitability for *D. dendriticum*. These areas, further from watershed regions and closer to dense rainforest zones, showed an increased likelihood of parasite presence. Similarly, in the mild and humid climate of Gilan Province, especially central regions, demonstrated the highest suitability. This may be attributed to the balanced combination of vegetation density, slope, and wetlands in the area, which together create favorable conditions for the survival and transmission of the parasite.

When comparing these findings with those of Khan et al. (2023) in the Himalayan ranges of Pakistan, notable differences emerge in the influence of bioclimatic variables. While the present study identified NDVI as the most influential variable, followed by Bio11, across the cold and semi-arid, warm and hyper-arid, and cool and sub-humid climates, Khan et al. (2021) reported Bio2 and Bio12 as the most significant climatic variables affecting the prevalence of dicrocoeliasis in the Himalayan region. These discrepancies highlight the complex interplay between topographic and bioclimatic factors in shaping parasite distribution across diverse climatic zones.

Conclusion

This study provides valuable insights into the prevalence and spatial distribution of *Dicrocoelium* infection in sheep across Iran's diverse climatic zones. By integrating prevalence data with bioclimatic and topographic variables, the study elucidates the ecological factors governing parasite distribution. The use of spatial modeling techniques, such as MaxEnt, highlights the critical role of vegetation cover, slope, and climatic variables in shaping the spatial patterns of dicrocoeliasis. The findings have significant implications for developing targeted intervention strategies and promoting sustainable livestock management. Future research should focus on refining predictive models by incorporating additional environmental and anthropogenic factors, thereby enabling more effective control measures to mitigate the economic impact of this parasitic disease.

Ethical Considerations

Compliance with ethical guidelines

This study involved no experiments on humans or animals. Therefore, no ethical approval was required.

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Authors' contributions

Conceptualization, supervision and funding acquisition: Behnam Meshgi; Methodology and validation: Ahmad Ali Hanfi Bojd; Data curation and analysis: Shirin Khodabakhsh Arbat. Software, formal analysis and writing the original draft, Abbas Oliya Ardekani; Investigation, resources, project administration, review and editing: Seyed Hossein Hosseini.

Conflict of interest

The authors declared no conflict of interest.

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