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RESEARCH ARTICLE

Spatial Risk Prediction and Serological Validation of Emerging Wild Boar-Borne Diseases in Eastern Heilongjiang, China

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ABSTRACT

Wild boars (*Sus scrofa*) have recently been identified as significant reservoirs and amplifiers of various emerging zoonotic viruses. In northeastern China, the increasing overlap between wild boar habitats, livestock farming areas and human settlements has raised serious concerns about cross-species transmission and spatial spillover of wild boar-borne infectious diseases. This study aimed to define the ecologically suitable distribution of wild boars and associated viruses, identify spatial hotspots of emerging wild boar-borne disease risk and validate the circulation of these pathogens within wild boar populations in high-risk areas. The goal was to provide a scientific basis for surveillance and transboundary prevention in ecologically sensitive regions. The MaxEnt ecological niche model was applied to predict the suitability of wild boars and spatial risk of disease emergence. A fuzzy overlay analysis was then conducted to delineate high-risk zones for wild boar-borne disease emergence. Within these high-risk zones, least-cost path modeling was used to analyze ecological connectivity and to identify major transmission routes. Between January 2023 and December 2024, a total of 158 wild boar serum samples were collected along the identified transmission routes. ELISA assays were used for serological testing and antibody prevalence was calculated. The MaxEnt model showed high predictive accuracy for wild boar distribution (AUC = 0.810, Kappa = 0.816) and for disease risk mapping (AUC = 0.818, Kappa = 0.803). The total area of high-risk zones for emerging wild boar-borne diseases reached 12,188.68 km², mainly located in Mudanjiang, Jixi, and Shuangyashan city. Three major transmission routes were identified within these regions and all converging in Hulin County. This area is characterized by semi-free-range hybrid wild boar farming and strong landscape connectivity, making it a critical node for potential virus spread. Serological testing confirmed PRV antibodies in 8.86% (14/158) of samples and PCV2 antibodies in 5.06% (8/158). The highest PRV antibody prevalence (21.05%, 95%CI: 9.55-37.32) was observed in Hulin County, suggesting its central role in local virus maintenance and transmission. This study proposes a four-stage spatial framework: habitat modeling, risk mapping, routes identification and serological validation for comprehensive assessment of wild boar-borne disease risks. It provides both theoretical and practical value for targeted surveillance and transboundary disease preparedness in ecologically sensitive regions

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INTRODUCTION

Wildlife hosts play increasingly prominent roles in the emergence of viral zoonoses, and wild boars (Sus scrofa) have been implicated as key reservoirs of veterinary and zoonotic viruses. With a rapidly expanding geographic range and frequent contact with domestic livestock and humans, wild boars represent critical nodes in the ecology of transboundary diseases. On the global scale, the rapid growth and spatial expansion of wild boar populations, driven by climate change and land-use transformation has created favorable conditions for pathogen persistence, amplification and cross-border transmission. Over the past decade, multiple studies have identified wild boars as asymptomatic or subclinical carriers of viruses such as African swine fever virus (ASFV), classical swine fever virus (CSFV), pseudorabies virus (PRV), porcine circovirus types 2 and 3 (PCV2/3), hepatitis E virus (HEV), rabies virus (RV) and canine distemper virus (CDV) (Meng, 2013; Liu *et al.*, 2021; Acosta *et al.*, 2022).

In Europe and Northeast Asia, ASFV is widely detected in wild boar carcasses, with passive surveillance indicating much higher positivity in carcasses than in hunted animals (Denstedt *et al.*, 2021). After ASFV spread into China in August 2018, genotype II was rapidly detected across more than 20 provinces (Zhao *et al.*, 2019), yet serological surveys of ASFV antibodies in Chinese wild boars remain largely absent. This rapid continental-scale spread underscores how global disease pressure can translate into acute regional vulnerability, particularly in areas where systematic wildlife surveillance remains insufficient. As such, Heilongjiang provides a critical lens through which to examine how global drivers of disease emergence interact with local ecological and surveillance constraints. By contrast, sero-epidemiological data for other viruses such as HEV, CDV and RV have been reported from different regions of China, indicating that wild boars can sustain substantial levels of viral exposure and may contribute to the circulation of multiple pathogens (Wu *et al.*, 2022; Wang *et al.*, 2023; Wang *et al.*, 2024).

Heilongjiang Province, which is in northeastern China and borders far Eastern Russia contains one of China's largest continuous forest ecosystems, providing optimal conditions for wild boar habitats and pathogen persistence. Moreover, its position along major transboundary wildlife movement corridors render the region particularly susceptible to the introduction and onward spread of emerging infections. This region acts as a critical intersection where global disease dynamics, ecological changes, and wildlife movement converge, making it especially vulnerable to cross-border pathogen transmission. Climate change, forest exploitation and changing land use patterns have contributed to altered wild boar behavior, including increased migration and expanding range boundaries (Zhang *et al.*, 2024). However, no published study has systematically described the infection status of wild boar-borne diseases in this region, and the spatial distribution of viral exposure risk and the role of ecological connectivity in promoting disease maintenance and spread remain poorly understood.

To fill this significant research gap, we established a spatially explicit analytical framework that integrates ecological niche modeling with multi-pathogen risk prediction (Bosch *et al.*, 2014; Lim *et al.*, 2022; Li *et al.*, 2022) and field-based serological validation to investigate the spatial epidemiology of emerging infectious diseases in wild boar populations of eastern Heilongjiang Province. This approach enabled the identification of suitable wild boar habitats, the spatial distribution of viral infection risks and the ecological connectivity among high-risk zones. Furthermore, model predictions were validated through serological surveys conducted across the study area. By combining spatial modeling with ground-truth data, this study provides a comprehensive understanding of disease dynamics in a transboundary context and offers a scientific basis for targeted wildlife disease surveillance and proactive cross-border risk mitigation strategies.

MATERIALS AND METHODS

Study area: The eastern region of Heilongjiang Province lies between 125.24°–135.07°E and 43.43°–48.48°N. It covered an area of approximately 136,900Km² and encompassing 8 cities, including Yichun, Hegang, Jiamusi, Shuangyashan, Qitahe, Jixi, Mudanjiang and Harbin (Fig. 1). The landscape dominated by plains and mountains is characterized by temperate forests interspersed with wetland ecosystems, providing optimal habitats for wild boars and contributing to pathogen persistence. According to the provincial catalogue, the region hosts approximately 476 nationally protected wildlife species, including 17 under Class I and 65 under Class II protection with representative species such as the Amur tiger, sable, wild boar, red deer and brown bear. This rich biodiversity not only enhances the ecological value of the region but also provides a vital foundation for scientific research.

Wild boar and wild boar-borne diseases occurrence points collection and pre-processing: It has been combined field investigations with multiple data sources to obtain comprehensive spatial distribution data from 2010-2024 on wild boars and wild boar-borne diseases in eastern Heilongjiang Province. These included published domestic and international literature, media reports, the Global Biodiversity Information Facility (https://www.gbif.org/) and records from local health authorities. Cross-checked reports from different sources and retained only cases consistently documented across multiple channels to ensure data reliability. Underreporting and misdiagnosis due to symptom overlap challenged data accuracy. So only laboratory-confirmed cases were included to minimize uncertainties.

In total, 268 distribution records of wild boars and 109 records of associated emerging infections, such as hepatitis E virus (HEV), porcine parvovirus (PPV), African swine fever (ASF), pseudorabies virus (PRV) and porcine circovirus type 2 (PCV2) were complied. To address spatial autocorrelation (SAC), the dataset was Geographic processed and spatial analysis using SDM Toolbox v1.1c within ArcGIS 10.8 (https://developers.arcgis.com/). A spatial filtering procedure was applied to maintain a minimum distance of 5km between occurrence points to reduce geographic sampling bias. The dataset was projected to the UTM-WGS-1984 coordinate system, and the spatial resolution was resampled to 30 arc-seconds to ensure consistency for subsequent analyses.

Selection and processing of environmental variables: A total of 67 climate variables were extracted from the CHELSA v1.2 database (https://chelsa-climate.org/). The Human Influence Index was obtained from the Socioeconomic Data and Applications Center (SEDAC) (http://sedac.ciesin.columbia.edu/wildareas/), while land use and river network data were sourced from the Institute

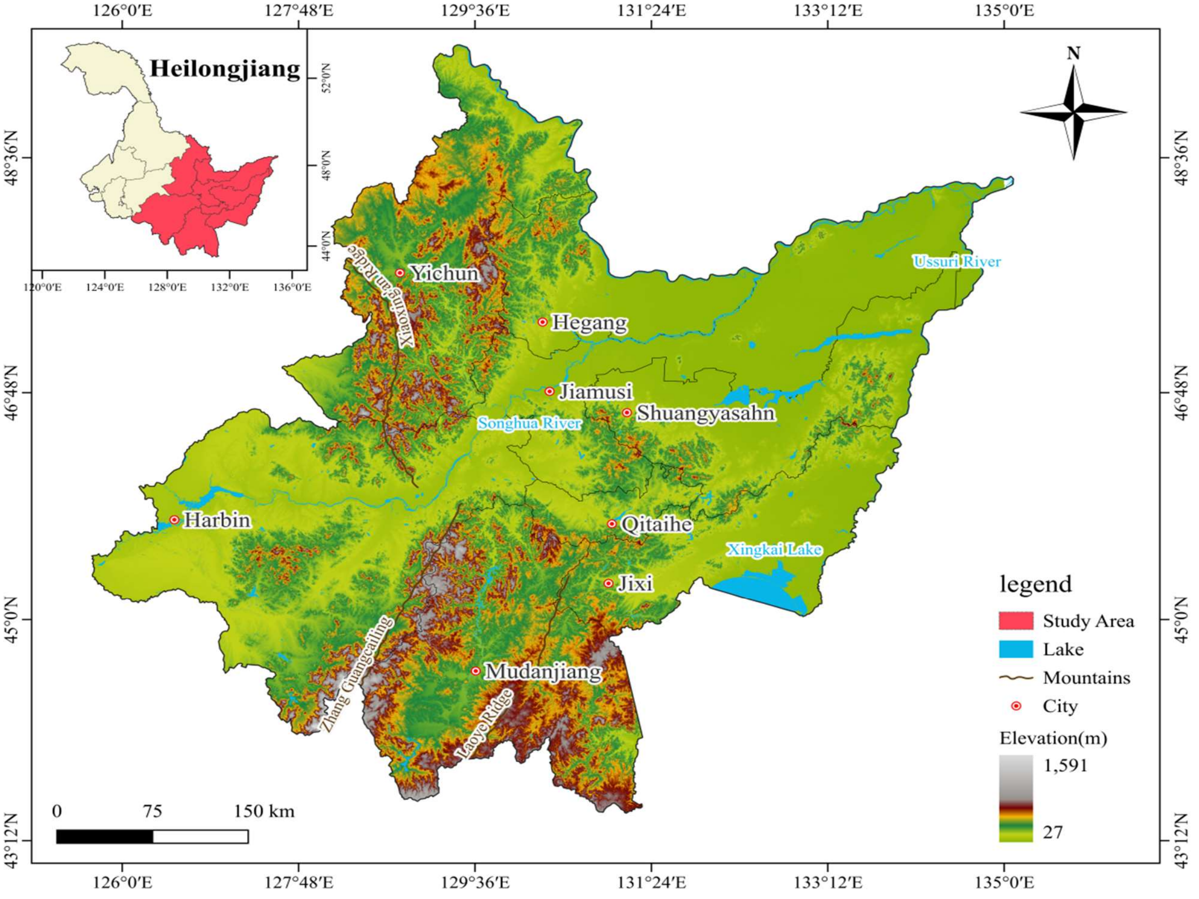

Fig. 1: Study area of the eastern region of Heilongjiang Province in China (Data source：The administrative boundary map of eastern Heilongjiang Province was obtained from the Resource and Environment Science Data Center of the Chinese Academy of Sciences (https://www.resdc.cn/.)

of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences (https://www.resdc.cn). Slope and elevation data were derived from 100-meter resolution digital elevation models (DEMs) provided by the Computer Network Information Center of the Chinese Academy of Sciences and the Global Science Data Hub (http://www.gscloud.cn). Surface solar radiation data were accessed from the National Tibetan Plateau Data Center (https://data.tpdc.ac.cn/home). All spatial layers were resampled to a uniform resolution of 30 arc-seconds (approximately 1km) using ArcGIS to ensure consistency across datasets.

The variable selection followed the method proposed by Liu *et al.* (2024) to reduce multicollinearity and avoid overfitting in the modeling process. Environmental variable preparation and supplementary analysis were performed with ENMTools version 1.0.5 (http://ydl.oregonstate.edu/pub/cran/web/packages/ENMTools/index.html). All environmental layers were imported into ArcGIS and Pearson correlation analysis was performed using the multivariate analysis tool. For variable pairs with a high correlation coefficient (|R| > 0.85), only the variable with a greater contribution or higher permutation importance in preliminary MaxEnt runs was retained. The Variance Inflation Factor (VIF) analysis was conducted using linear regression to identify multicollinearity between continuous and categorical variables. Variables with a VIF greater than 10 were excluded to ensure that only independent predictors remained in the final model. This approach ensured that the final model included only ecologically relevant and non-redundant predictors.

Modeling building

Parameter optimization for MaxEnt model construction: MaxEnt version3.4.4 (http://biodiversityinformatics.amnh.org/open_source/MaxEnt/) was used to build the ecological niche models. Because relying on default parameters can lead to overfitting and inaccurate predictions (Fernández and Morales, 2019; Zhao *et al.*, 2022), we optimized the feature classes (linear, quadratic, hinge, product and threshold) and the regularization multiplier (0.1–4, increment 0.1) using the Kuenm package (Cobos *et al.*, 2019). Model fit and complexity for each parameter combination were evaluated using the corrected Akaike Information Criterion (AICc), the area under the receiver operating characteristic curve (AUC), the 10% training omission rate and the minimum training presence omission rate (Gutierrez and Heming *et al.*, 2018; Ouyang *et al.*, 2022), and the optimal parameter combination was identified based on these integrated performance metrics. Through this systematic optimization process, it has been significantly enhancing the predictive performance of the model, ensuring that the results are both scientifically rigorous and practically applicable.

Habitat suitability modeling: The de-redundant occurrence data and the screened environmental variables were imported into MaxEnt with 80% of the occurrence records used for training dataset and the remaining 20% for testing dataset. Model output was set to the logistic format to obtain the probability of habitat suitability. The jackknife test was applied to evaluate the contribution and permutation importance of each environmental variable. The model ran ten times in subsample mode and the mean prediction from these runs was used to improve stability and consistency.

Model validation: Model performance was evaluated using receiver operating characteristic (ROC) curves and the area under the curve (AUC) (Ahmadi *et al.*, 2023). The Kappa statistics were also calculated using the Presence Absence package in R to further assess model accuracy, and both indices were interpreted following published criteria (Dongyang *et al.*, 2023; Shi *et al.*, 2025).

Classification of habitat suitability levels: The predicted habitat suitability maps were converted to raster format using Conversion tool in ArcGis 10.8, and the Reclassify tool was then used to divide the raster data into suitability levels (Ikhumhen *et al.*, 2020). The classification breakpoints were determined using three thresholds, namely the training sensitivity sum (TSS) threshold, the MTSPS threshold and the true presence threshold (TPT) (Zhao *et al.*, 2025).

Prediction of high-risk areas for wild boar-borne diseases: The Fuzzy Overlay function within the Spatial Analyst tool of ArcGIS 10.2 was used to identify high-risk areas. The habitat suitability map of wild boar and the distribution risk map of wild boar-borne diseases in eastern Heilongjiang Province were overlaid by selecting the "AND" option in Fuzzy Overlay (He *et al.*, 2024). This approach ensured that an area was classified as high risk only when all considered environmental and climatic factors consistently indicated elevated risk.

Connectivity analysis within high-risk areas for wild boar-borne diseases

Construction of the resistance layer: To construct the resistance layer for assessing cross-border transmission risk, vegetation and elevation were selected as key environmental factors based on movement patterns and foraging habits of wild boars. Vegetation density influences both food availability and the level of cover, thereby directly shaping habitat suitability. Elevation affects movement potential, as variations in terrain and altitude influence accessibility and the likelihood of long-distance dispersal.

Each vegetation and elevation grid cell was assigned a corresponding resistance value and converted into a classified layer. Vegetation data was already available in classified form, whereas elevation data were reclassified in ArcGIS based on known habitat preferences of wild boars. Resistance values ranging from 1 to 9 were assigned to different habitat types using an expert-based evaluation, with lower values (e.g., 1) indicating wild boar resistance and greater ease of movement, and higher values (e.g., 9) representing maximum resistance and limited movement potential (Carroll *et al.*, 2020).

Finally, the vegetation and elevation layers were integrated using the Weighted Overlay tool in ArcGIS 10.8 (Carroll *et al.*, 2020). The resulting composite resistance layer provides a robust spatial basis for analyzing wild boar movements within high-risk areas and offers valuable support for subsequent studies on disease transmission dynamics.

Calculation of the LCP: The Least Cost Path (LCP) model was used to predict potential migration routes of wild boars within high-risk areas (Özcan *et al.*, 2020). Land cover and elevation were reclassified using the Jenks natural breaks method and used as cost factors, and resistance values from 1 (lowest) to 9 (highest) were assigned according to wild boar activity preferences and landscape characteristics such as vegetation density, human infrastructure and topography. The values represent the relative permeability of different landscape features to wild boar movement, with lower values indicating higher permeability and higher values indicating greater resistance. The resulting cost surface was combined with outbreak locations using the Grouping Analysis tool in ArcGIS 10.8 to construct LCPs model (Özcan and Erzin, 2020), and paths originating far from the border or already merged into primary routes were excluded.

Serological validation within high-risk areas for wild boar-borne diseases

Serum collection: From January 2023 to December 2024, a total of 158 serum samples from hybrid wild boars were collected along potential high-risk transmission routes 1-3 of wild boar-borne diseases in eastern Heilongjiang Province. Capture devices were positioned above the wild boar's mouths to maintain a natural standing posture, which facilitated safe and accurate access to the blood vessels. Blood was drawn from the internal jugular vein using a 20mL syringe, and 5mL of whole blood was immediately processed with a portable centrifuge at 3,000×g for 10 minutes to separate the serum. The serum samples were stored at −20°C until further analysis.

Serological analysis: Serum samples were analyzed using a commercial ELISA kit in accordance with the manufacturer's protocol. The results were expressed as the inhibition rate of the optical density (%OD), calculated using the formula that was $\% \text{ OD} = 100 \times (S–N)/(P-N)$, where S denotes the sample reading, N and P are the optical density values for the negative and positive controls, respectively. Based on the assay criteria, ELISA results were interpreted using specific %OD thresholds. For PRV antibody detection, samples with %OD values below 50 were classified as positive, values between 50 and 60 were considered doubtful, and values above 60 were classified as negative. For PCV2 antibody detection, %OD values ≥ 100 indicated a positive result, values between 15 and 100 were considered doubtful, and values ≤ 15 were deemed negative (Wang *et al.*, 2023).

Statistical methods: The serological prevalence was calculated as the proportion of positive samples relative to the total number of samples and expressed as a percentage. The true prevalence for both the overall dataset and spatial groupings was estimated using the AusVet Epitools software package (https://epitools.ausvet.com.au/) (Lishchynskyi *et al.*, 2022). Associations between apparent prevalence and factors such as location, age, and sex were assessed using Fisher's exact test with statistical significance determined at $P \leq 0.05$. For all estimates, 95% confidence intervals were calculated and reported.

RESULTS

Habitat Suitability Modeling

Determination of occurrence data and environmental variables: This approach retained the most informative records while minimizing unnecessary exclusions, resulting in 213 occurrence points for wild boar and 78 records for wild boar-borne diseases after processing.

Correlation analysis was first used to select 12 environmental variables for modeling wild boar distribution and 10 variables for modeling wild boar-borne diseases (Fig. 2). The importance of each variable was then assessed in the MaxEnt model using the jackknife test and variables with a contribution rate greater than 5 were identified as key predictors. The collinearity analysis for these environmental variables revealed VIF values between 1.395 and 7.487, all of which were below the commonly accepted threshold of 10, suggesting that there was no indication of multicollinearity present. Finally, the variables of hf, bio15, bio3, prec5, alt, and bio7 were retained as key predictors in the wild boar distribution model (Table 1); In the modeling of wild boar-borne diseases, although the contributions of rs, bio16, and prec07 were all less than 5%, but these indispensability was notable as removing these variables significantly reduced the model's gain value. Therefore, these variables must be retained. The collinearity tests for these variables indicated VIF values between 2.548 and 8.437, all falling below the threshold of 10, thereby confirming that no multicollinearity issues were present. Ultimately, hf, dis.river, bio03, rs, bio16, and prec07 were identified as the key variables for the modeling of wild boar-borne diseases (Table 1).

Table 1: Analysis of environmental variables contribution rates of wild boar and wild boar-borne diseases

Category	Variable	Percent contribution (%)	Permutation importance (%)	VIF
wild boar	Hf	32.8	21.7	1.395
	bio15	22.2	9.9	2.458
	bio03	10.8	10.2	7.487
	prec05	7.1	17.7	4.566
	Alt	6	7.7	6.35
	bio7	5.9	2.2	5.671
wild boar–borne infectious diseases	Hf	73.7	52.5	6.324
	dis.river	8	9.6	3.475
	bio03	5.2	13.9	2.548
	Rs	4.1	2.3	4.88
	bio16	2.5	6.6	8.437
	prec07	2.2	0.5	5.231

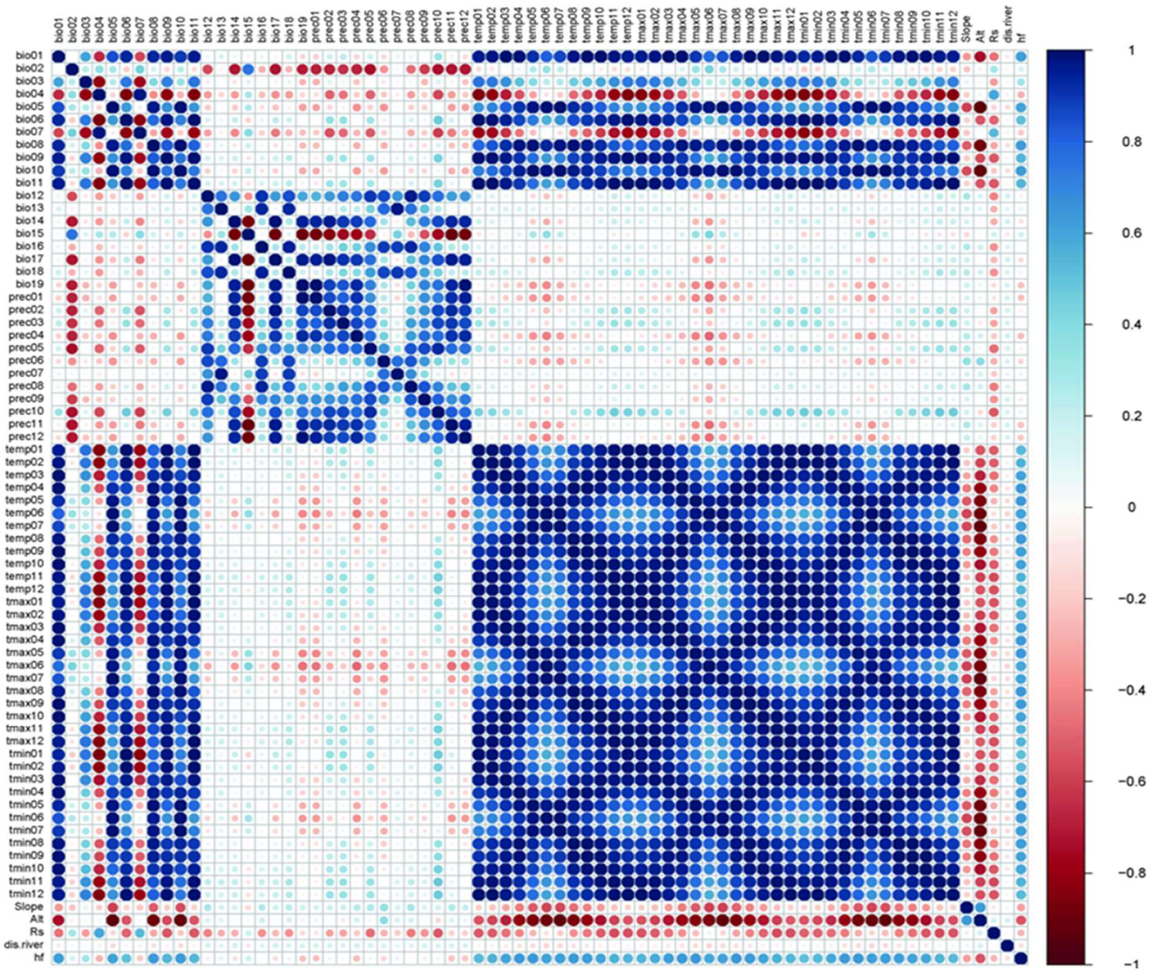

Fig. 2: Correlation heatmap of environmental variables. The figure illustrates the correlation coefficients between various environmental variables. The color scale ranges from −1 (indicating strong negative correlation and shown in red) to +1 (indicating strong positive correlation and shown in blue). Correlation coefficients near 0 represent weak or no correlation. To avoid overfitting, for pairs of variables with a correlation coefficient |R| > 0.85, one variable is selected based on its stronger association with the geographical distribution of wild boar and the wild boar-borne diseases. This approach ensures the robustness and accuracy of the model's predictions by prioritizing the most relevant variables.

Model evaluation: The model parameter with FC = lph and RM = 0.7 was chosen for wild boar distribution modeling (Fig. 3-A), while the model parameter with FC = lpth and RM = 1.2 was selected for modeling the distribution of wild boar-borne diseases (Fig. 3-B).

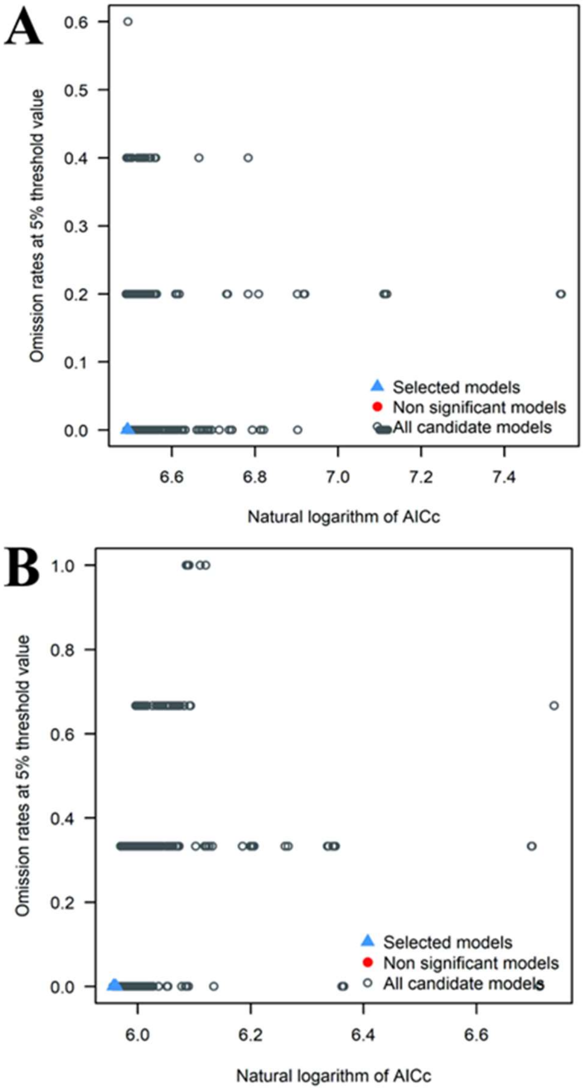

Fig. 3: Scatter plots of optimal MaxEnt models. (A)The candidate MaxEnt models for wild boar distribution;(B). The candidate MaxEnt models for wild boar-borne emerging infectious disease distribution. A total of 1,160 candidates MaxEnt models under different combinations of FC and RM parameters were ran using the Kuenm package based on the distributions of wild boars and emerging wild boar-borne diseases. The blue triangles mark the optimal model that met the criteria of statistical significance, an omission rates below 5% and delta AICc values less than 2. The red circles indicate non-significant models and grey circles represent all candidates models.

Model validation demonstrated strong predictive performance with an AUC value of 0.810 (SD=0.175) and Kappa value of 0.816 for the distribution model of wild boar, indicating that the model prediction results are reliable (Fig. 4-A); Similarly, the distribution model of wild boar-borne diseases achieved a high AUC value of 0.818 (SD=0.120) and Kappa value of 0.803, suggesting that the model performed well and consistent accuracy (Fig. 4-B).

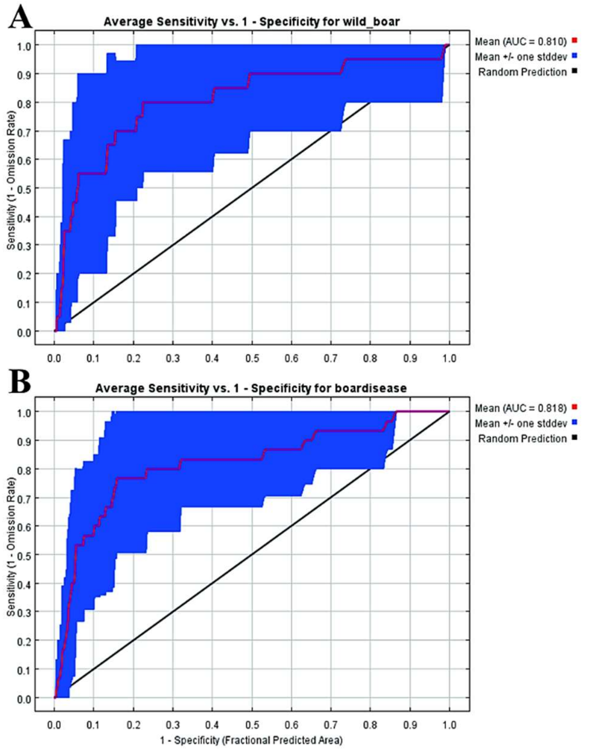

Fig. 4: The receiver operating characteristic (ROC) curve assessing the predictive performance of distribution models. (A) The performance of wild boar distribution model with an AUC of 0.810; (B) The performance of wildboar-borne diseases distribution model with an AUC of 0.818. High AUC values indicate that the model performed well and consistent accuracy in differentiating between suitable and unsuitable habitats.

Spatial Risk Mapping

Model Prediction: Medium-high suitability areas for wild boar were concentrated in the central and eastern study region, mainly in Jixi, Shuangyashan, Jiamusi, Harbin and Mudanjiang, with smaller patches in Yichun and Hegang. The total area of high suitability was 14,220.43 km², medium suitability 19,411.81 km² and low suitability 92,066.92 km². The total area of medium-high suitability accounts for 14.99% of the entire study are (Fig. 5-A); for wild boar-borne diseases, medium-high risk zones were mainly in the south and west, particularly in Jixi, Mudanjiang and Shuangyashan city, with smaller areas in Jiamusi. The total area of high risk was 12,188.68 km², medium risk 7,200.18 km² and low risk 54,150.80 km².The total area of medium-high risk zones accounts for 8.64% of the entire study area (Fig. 5-B).

Spatial distribution maps of habitat suitability are shown for (A) wild boar and (B)wild boar-borne diseases. Suitability levels were classified into four categories based on the TSS training sensitivity threshold, MTSPS threshold and TPT balance threshold: unsuitable ($P \leq 0.1542$, gray), low ($0.1542 < P \leq 0.40$, orange), medium ($0.40 < P \leq 0.5639$, green) and high ($P > 0.5639$, dark blue). Higher suitability levels indicate a greater likelihood of wild boar presence and an elevated risk of disease transmission. Fitting analysis with occurrence records revealed that most sample points were located within medium–high suitability areas.

Response Curve Analysis: MaxEnt modelling identified 12 environmental variables influencing the distribution of wild boar and wild boar-borne diseases. To further determine the threshold ranges of these variables in the areas most suitable for wild boar, we set a high suitability index of 0.5639 to extract the corresponding variable ranges, which showed that optimal wild boar distribution occurs when bio03 <159, hf > 48, prec05 > 56 mm, bio15 < 82, elevation (alt) ranges from 73 to 144 m and bio07 is between 47.7 and 49.5°C. These thresholds indicate that wild boars preferentially occupy environments with relatively stable thermal conditions, moderate precipitation and intermediate elevations, which are likely to reduce physiological stress while ensuring adequate vegetation productivity and food availability. The positive association with human footprint values above 48 suggests that wild boars frequently utilize landscapes with moderate human disturbance, such as forest–farmland interfaces, where anthropogenic food sources and natural cover coexist.

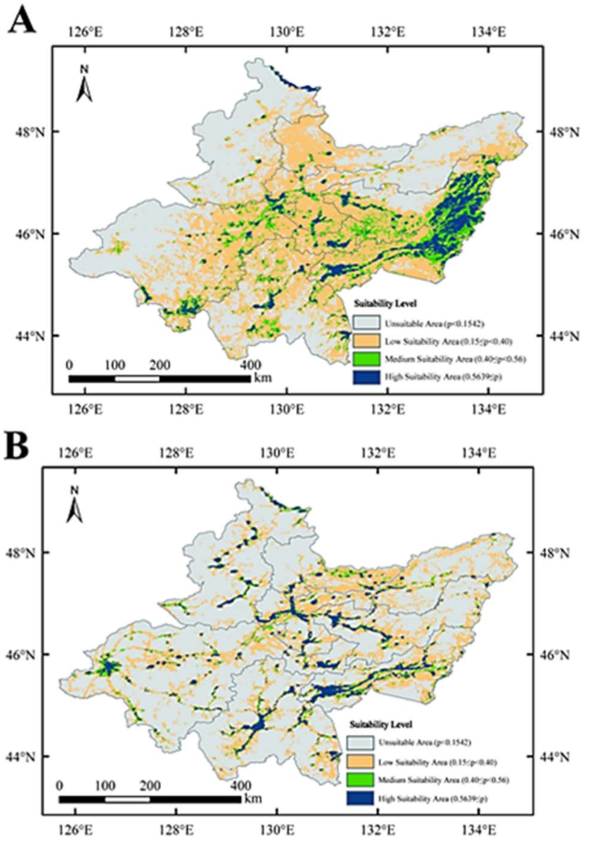

Fig. 5: Predicted habitat suitability for wild boar and risk distribution of wild boar-borne diseases in eastern region of Heilongjiang Province. Spatial distribution maps of habitat suitability are shown for (A) wild boar and (B)wild boar-borne diseases. Suitability levels were classified into four categories based on the TSS training sensitivity threshold, MTSPS threshold and TPT balance threshold: unsuitable (P≤0.1542, gray), low (0.1542 <P≤0.40, orange), medium (0.40 <P≤0.5639, green) and high (P>0.5639, dark blue). Higher suitability levels indicate a greater likelihood of wild boar presence and an elevated risk of disease transmission. Fitting analysis with occurrence records revealed that most sample points were located within medium–high suitability areas.

The suitability index threshold at 0.3887 was set to define highly risk transmission regions for wild boar-borne diseases. The extracted variable ranges indicate that optimal risk distribution occurs when the bio03<160, bio16<95 mm, dis. river<0, hf >100, prec07<80 mm and rs<175 W/m² (Fig. 6 and Fig. 7). Ecologically, these conditions may reflect environments where pathogens can persist more effectively and where contact rates among wild boars, water sources and human-influenced landscapes are elevated. Proximity to rivers may facilitate indirect transmission through contaminated water or shared aggregation sites, while higher human activity levels may increase opportunities for spillovers between wild boars and domestic pigs.

Ecological Connectivity Analysis: The central and eastern parts of eastern Heilongjiang Province are considered high-risk areas for emerging infectious diseases carried by wild boar. These areas include the western part of Jiamusi and Shuangyashan regions, the central part of Qitaihe and Mudanjiang region, and most of Jixi. All these areas face a high-risk outbreak of wild boar-borne emerging infectious diseases. In contrast, regions such as Yichun, Harbin and the eastern part of Jiamusi are classified as moderate to low-risk areas for these diseases (Fig. 8).

In this study, three potential migration routes for wild boar-borne emerging infectious disease transmission were identified within the high-risk zones, as shown in Fig. 8: Route 1 passes through Jixian county, Youyi county and Baoqing county in Shuangyashan city and Hulin county in Jixi city; Route 2 runs through Qiezihe county in Qitaihe City, Baoqing county in Shuangyashan city and Hulin county in Jixi city; Route 3 goes through Mishan county and Hulin county in Jixi City. These three routes feature dense vegetation and abundant water, creating optimal habitats for wild boar and facilitating pathogen spread. As a result, wild boar-borne emerging infectious diseases are highly likely to occur along these routes. It is important to note that all three routes pass through Hulin County in Jixi city. Hulin county is a major high-risk area for wild boar-borne diseases in eastern Heilongjiang Province and hosts many hybrid wild boar farms, where resource sharing between wild and domestic pigs promotes cross-species disease transmission.

Three primary transmission routes (Routes 1-3) identified via least-cost path (LCP) model to predict potential wild boar-borne emerging infectious disease transmission corridors within high-risk areas. Suitability levels were classified into four categories based on the TSS training sensitivity threshold, MTSPS threshold and TPT balance threshold: unsuitable (P≤0.1542, gray), low (0.1542 <P≤0.40, orange), medium (0.40 <P≤0.5639, green) and high (P>0.5639, dark blue). Higher suitability levels indicate high-risk areas for disease transmission. All corridors converge at Hulin County (Jixi City), a critical transmission zone containing hybrid wild boar farms.

Serological Validation: A serological survey was conducted and antibodies of PRV and PCV2 was detected in three major transmission routes within the high-risk areas in the eastern part of Heilongjiang Province，China. The total seroprevalence of PRV was 8.86% (14/158, 95% CI 4.93-14.42) and the estimated overall true prevalence was 8.83% (95% CI 4.89-14.97) (Table 2). For PCV2, the total seroprevalence was 5.06% (8/158, 95% CI 2.21-9.73) and the estimated overall true prevalence was 4.57% (95% CI 1.78-9.74) (Table 3).

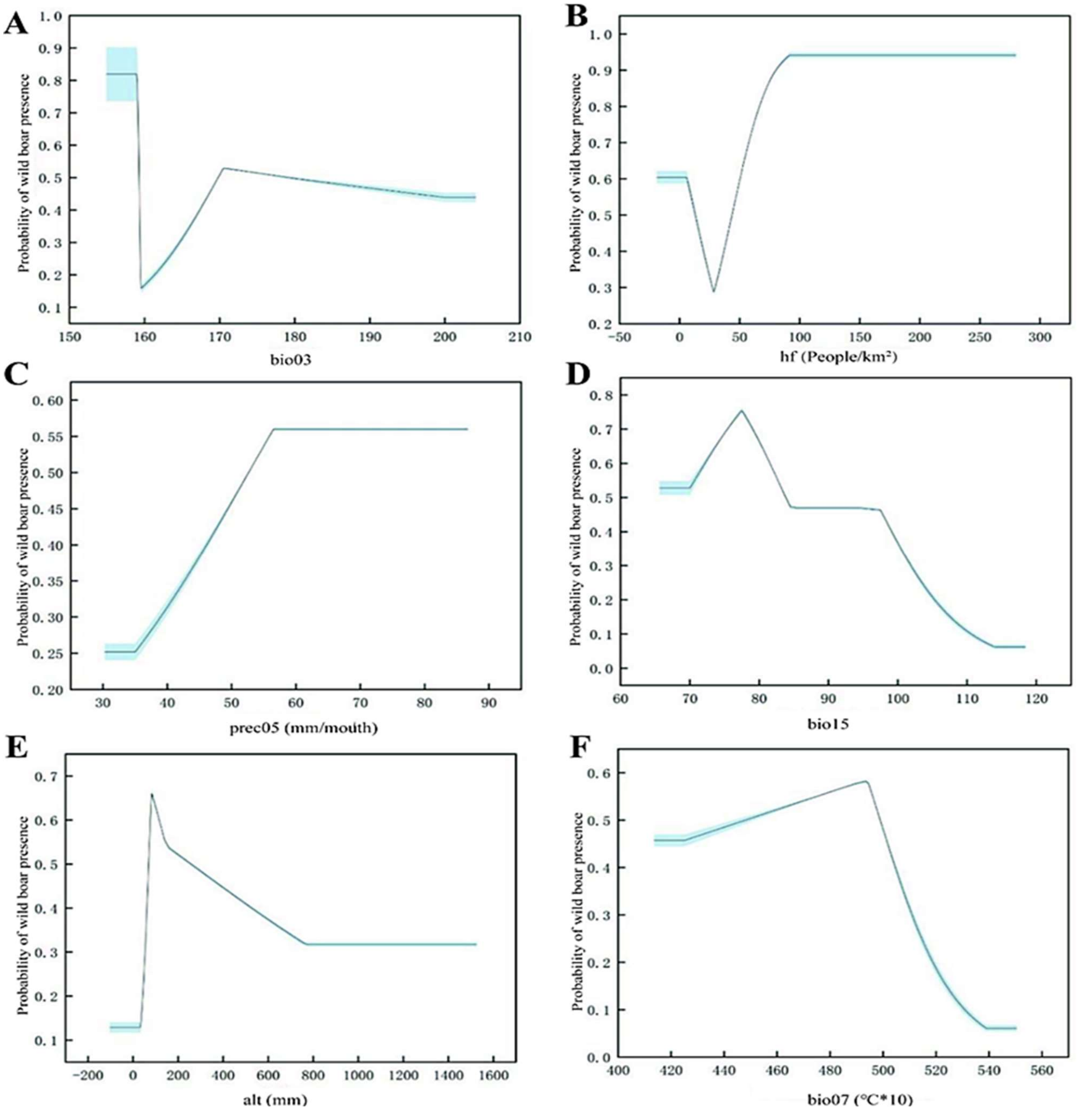

Fig. 6: Response curves of key environmental variables influencing wild boar habitat suitability based on the MaxEnt model. These patterns indicate the optimal environmental variables ranges for wild boar habitat suitability. The black lines represent the mean response curves generated from 10 replicates of the MaxEnt model, and the light blue shading indicates the range of variation across replicates. (A) Isothermality index (bio03): The suitability probability increases sharply when bio03 exceeds 160 and peaks at 171, then declines gradually; (B) Human activity influence index (hf): The suitability probability rises rapidly when hf exceeds 28 and reaching a maximum at 91 before stabilizing; (C) Precipitation in May (prec05): The suitability probability increases steeply and peaking at 56 mm, then remains stable; (D) Precipitation seasonality coefficient (bio15), (E) elevation (alt) and (F) annual temperature range (bio07) all show an increase in suitability to respective peaks of 77, 82 m, and 49.3 °C then followed by a decline.

Table 2: Seroprevalence of PRV antibodies in hybrid wild boars from three potential high-risk transmission routes in the eastern region of Heilongjiang Province, China

Variable	City	County	Sample size (n)	Sero-positive (n)	Apparent prevalence (95%CI)	True prevalence (95%CI)	Fisher's exact p
City	Shuangyashan	Jixian	27	0	-	-	0.385
		Youyi	19	2	10.53% (1.3,33.14)	10.7% (2.18,34.15)	
		Baoqing	31	2	6.45% (0.79,21.42)	6.13% (0.88,22.16)	
	Jixi	Hulin	38	8	21.05% (9.55,37.32)	22.53% (11.32,39.71)	
		Mishan	15	2	13.33% (1.66,40.46)	13.86% (3.07,41.44)	
	Qitaihe	Qitaihe	17	0	-	-	
		Qiezihe	11	0	-	-	
Gender	Female		127	10	6.3% (2.76,12.03)	5.95% (2.5,12.29)	0.478
	Male		31	4	19.35% (7.45,37.47)	20.62% (9.2,39.64)	
Age (Mouths)	>12		69	3	4.35% (0.91,12.18)	3.76% (0.55,12.38)	0.095
	<12		89	11	12.36% (6.33,21.04)	12.76% (6.79,22.24)	
Total			158	14	8.86% (4.93,14.42)	8.83% (4.89,14.97)	

Table 3: Seroprevalence of PCV2 antibodies in hybrid wild boars from three potential high-risk transmission routes in the eastern region of Heilongjiang Province, China

Variable	City	County	Sample size (n)	Sero-positive (n)	Apparent prevalence (95%CI)	True prevalence (95%CI)	Fisher's exact p
City	Shuangyashan	Jixian	27	0	-	-	0.381
		Youyi	19	0	-	-	
		Baoqing	31	0	-	-	
	Jixi	Hulin	38	3	7.89% (1.66,21.38)	7.75% (1.93,22.25)	
		Mishan	15	0	-	-	
	Qitaihe	Qitaihe	17	3	17.65% (3.8,43.43)	18.7% (5.83,44.98)	
		Qiezihe	11	2	18.18% (2.28,51.78)	19.31% (4.65,52.47)	
Gender	Female		127	6	4.72% (1.75,10)	4.18% (1.33,10.03)	0.656
	Male		31	2	6.45% (0.79,21.42)	6.13% (0.88,22.16)	
Age (Mouths)	>12		69	2	2.9% (0.35,10.08)	2.13% (0.23,10.07)	0.467
	<12		89	6	6.74% (2.51,14.1)	6.45% (2.39,14.54)	
Total			158	8	5.06% (2.21,9.73)	4.57% (1.78,9.74)	

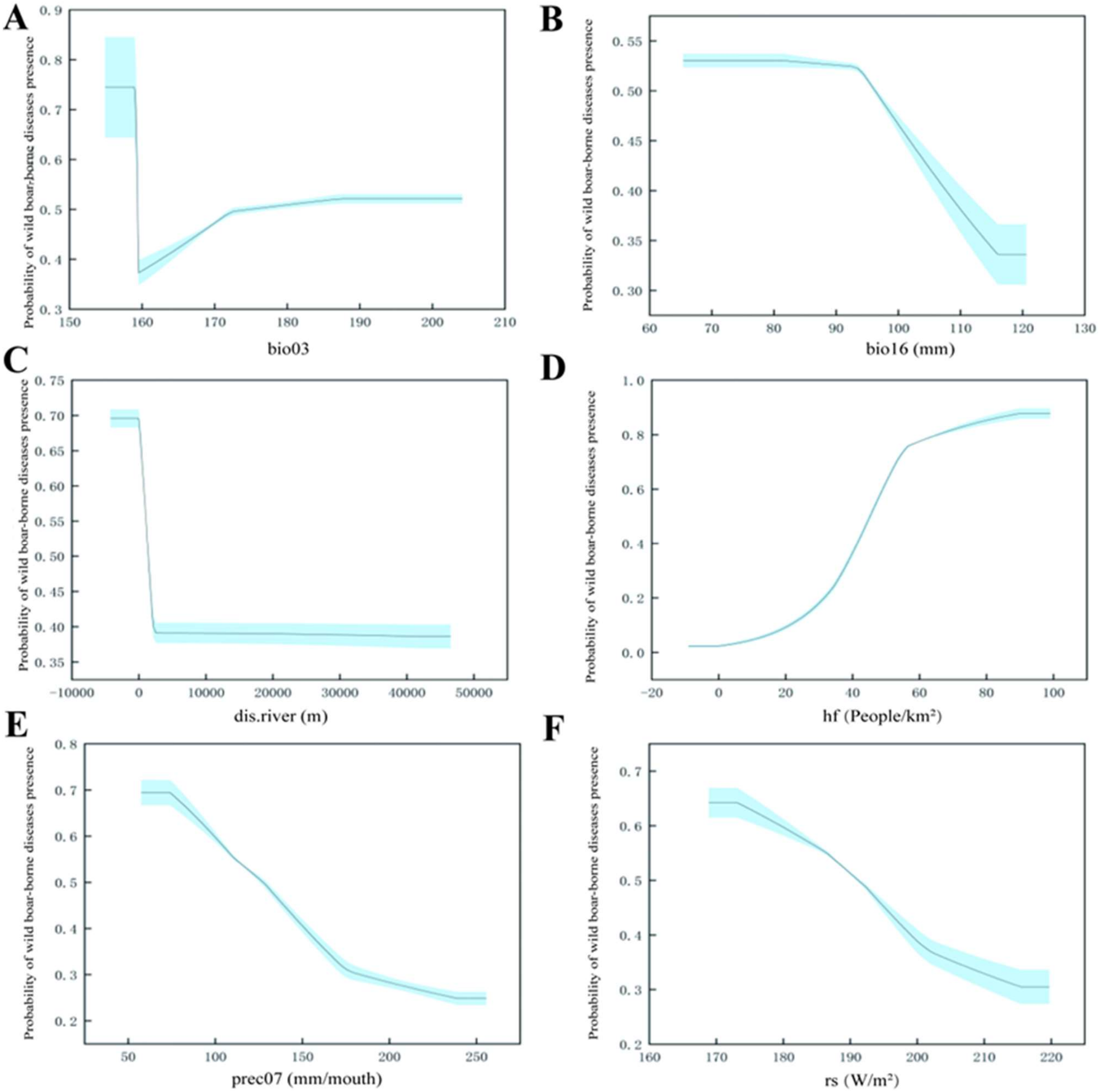

Fig. 7: Response curves of key environmental variables affects the predicted risk transmission areas of wild boar-borne diseases generated using the MaxEnt model. These results define the optimal environmental ranges associated with the greatest predicted suitability for wild boar–borne emerging infectious disease transmission risk. The black lines represent the mean response from 10 replicate runs and the light blue shading indicates variability among replicates. (A) Isothermality index (bio03): suitability increases when bio03 exceeds 160 and peaks at 172, then gradually declines; (B) Precipitation in the wettest month (bio16): suitability is highest between 70 and 95 mm, decreases sharply after 95 mm and stabilizes beyond 115 mm; (C) Distance to rivers (dis.river): suitability is highest at the shortest distances and declines before stabilizing with increasing distance. (D) Human activity influence index (hf): suitability increases markedly with higher hf values, peaking at 100. (E) Precipitation in July (prec07): suitability is highest when precipitation is below 100 mm, then declines with further increases. (F) Surface solar radiation (rs): suitability is highest below 180 W/m² and decreases steadily with higher radiation levels.

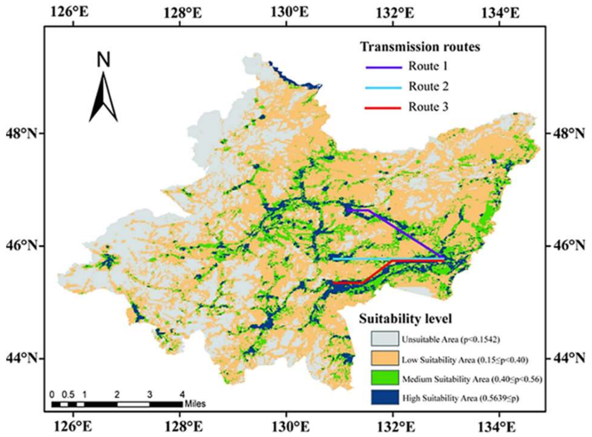

Fig. 8: Potential transmission routes and habitat suitability for wild boar-borne emerging infectious diseases in high-risk areas of eastern Heilongjiang Province. Three primary transmission routes (Routes 1-3) identified via least-cost path (LCP) model to predict potential wild boar-borne emerging infectious disease transmission corridors within high-risk areas. Suitability levels were classified into four categories based on the TSS training sensitivity threshold, MTSPS threshold and TPT balance threshold: unsuitable (P≤0.1542, gray), low (0.1542 <P≤0.40, orange), medium (0.40 <P≤ 0.5639, green) and high (P>0.5639, dark blue). Higher suitability levels indicate high-risk areas for disease transmission. All corridors converge at Hulin County (Jixi City), a critical transmission zone containing hybrid wild boar farms.

No PRV was detected in wild boars from Jixian County, Qitaihe city and Qiezihe county. However, PRV was detected in Youyi county, Baoqing county, Hulin county and Mishan county, with the highest seroprevalence observed in Hulin county at 21.05%; For PCV2, no cases were found in the three counties of Shuangyashan and Mishan county in Jixi, but positive results were detected in Hulin county, Qiezihe county and Qitaihe city, with the highest detection rate of 18.18% found in Qiezihe County. No significant associations were found between the occurrence of PRV and PCV2 and the city (P=0.385/0.381), gender (P=0.478/0.656), or age (P=0.095/0.467) (Tables 2 and Table 3)

DISCUSSION

This study integrated ecological niche modeling (MaxEnt), spatial risk prediction and field-based serological validation to develop a spatial framework for identifying high-risk areas and potential transmission corridors of wild boar-borne viral diseases in eastern Heilongjiang Province, China. The MaxEnt model showed strong performance in predicting both wild boar habitat suitability and wild boar-borne disease risk with AUC values of 0.810 and 0.818, respectively. These results are consistent with the widespread use of MaxEnt in wildlife disease ecology (Lim *et al.*, 2022; Díaz-Cao *et al.*, 2023). Areas with high habitat suitability particularly around Jixi and Shuangyashan, largely overlapped with areas of elevated disease risk. This spatial overlap suggests a strong correlation between ecological suitability and virus exposure. Such convergence between host habitat and pathogen risk reflects a well-documented pattern in zoonotic emergence, where favorable habitats for wildlife often align with viral amplification zones due to increased density and contact rates. Integrating these spatial layers is therefore critical for anticipating hotspots before outbreaks become apparent.

Key environmental variables influencing wild boar distribution included the hf, bio03, bio15 and prec05. These findings align with previous research, which suggests that wild boars prefer environments with moderate human disturbance, dense forest coverage and abundant water resources (Barrios-Garcia and Ballari, 2012; Bosch *et al.*, 2014). Fragmented forests and wetlands provide food and shelter for wild boars, promoting population aggregation and facilitating disease transmission. From a broader ecological perspective, such transitional zones, especially forest–agriculture mosaics create ecotones where multiple species converge, thereby increasing the probability of spillover events. These are often overlooked in classical veterinary surveillance, but modeling reveals them as high-risk interfaces.

In the risk model for wild boar-borne diseases, high-risk transmission environments were mainly driven by proximity to dis. river, rs, prec07 and hf. These humid and low-radiation environments are known to favor the persistence and environmental transmission of viruses, especially enveloped viruses such as PRV and PCV2, which are more stable in water, secretions and moist soil (Gillespie *et al.*, 2009). Response curves from this study further support these findings, showing that disease transmission potential increases in areas with solar radiation below 180 W/m2 and July precipitation below 100 mm. These environmental conditions likely support virus survival and spread in densely populated and shaded habitats (Nicastro *et al.*, 2021). This indicates a clear ecological mechanism whereby abiotic conditions indirectly affect transmission potential, reinforcing the need for ecological monitoring in zones with persistent surface moisture and limited UV exposure conditions that promote environmental viability of pathogens.

The LCP analysis identified three potential ecological corridors for virus transmission within the high-risk zones. These routes converge in Hulin City and form a critical corridor intersection. These corridors are characterized by low slope, high forest coverage and strong habitat connectivity, which is consistent with findings from other studies on movement pathways of forest-dwelling wildlife (Carroll *et al.*, 2020; Özcan and Erzin, 2020). Hulin is a transitional zone between wildlife habitats and human-dominated landscapes, where hybrid wild boars are raised in semi-free-range systems and share feed, water and even enclosures with domestic pigs. This shared interface significantly increases the risk of cross-species virus transmission (Meng, 2013). The spatial structure identified in this region supports the concept of a "wild boar ecological hub" proposed by (Bosch *et al.*, 2014) and aligns with Özcan's (Özcan and Erzin, 2020) "viral corridor" framework from central Turkey. These landscape-level corridors not only facilitate animal movement but also represent potential routes of pathogen flow, a concept increasingly emphasized in landscape epidemiology. Mapping and managing these corridors can provide preemptive disease containment pathways.

Serological testing further validated spatial predictions. A total of 158 serum samples were collected along the three ecological corridors. The overall seroprevalence of PRV was 8.86%, while PCV2 was detected in 5.06% of samples. Notably, PRV seropositivity in Hulin reached 21.05%, highlighting its central role in the regional virus transmission network. For comparison, previous studies have reported PCV2 seroprevalence in wild boars from Jiangxi and Sichuan provinces ranging from 5 to 26.5% (Hu *et al.*, 2022; Yang *et al.*, 2022), placing our findings at the lower bound. This may reflect the specific context of our study population—hybrid wild boars living along forest edges in border areas with lower levels of contact and exposure due to semi-free-ranging management and sampling focused on high-risk corridors.

In contrast, the PRV results are more concerning. Nationwide surveillance in domestic pig populations has shown PRV seroprevalence ranging from 12.36 to 25.04%, with levels below 10% reported in low-endemic provinces such as Jiangxi, Hunan, and Shaanxi (Zhao *et al.*, 2023). Except for Hulin, PRV seroprevalence in our study regions fell within this lower range, while Hulin's rate of 21.05% was comparable to areas of moderate PRV endemicity in domestic pigs. Internationally, wild boars in Russia's Far East have shown PRV seroprevalence as high as 32.5% (Kukushkin *et al.*, 2009), suggesting sustained environmental exposure. Although Hulin's rate is slightly lower than that, the presence of high ecological connectivity and close interaction between wild and domestic pigs in shared spaces may create conditions for viral amplification. Notably, PRV is known to spread efficiently through direct contact, vertical transmission and aerosols, especially under closed or semi-enclosed farming systems (Chen *et al.*, 2025).

Despite the strength of the findings, this study has some limitations. Serum samples were only collected from regions along high-risk corridors and may not represent the broader wild boar population in eastern Heilongjiang. Additionally, the study only tested PRV and PCV2 and did not include key pathogens such as ASFV or CSFV. Furthermore, the absence of molecular testing, such as PCR or viral genotyping means we cannot confirm whether detected strains originated from domestic pigs or wild reservoirs. Therefore, the serological data in this study primarily serves to validate spatial risk predictions rather than provide a full epidemiological profile. This approach is consistent with recent international efforts that integrate ecological models with field data to support targeted disease surveillance (Liu *et al.*, 2024). Such hybrid modeling-validation strategies are increasingly used in surveillance design under the 'One Health' framework, especially in regions with limited diagnostic resources but pressing wildlife–livestock interface concerns.

In conclusion, we recommend that Hulin and the surrounding ecological corridors be prioritized for future monitoring. A buffer zone should be established between wildlife activity areas and domestic pig farms to reduce contact. Strict management of hybrid wild boar farming systems is also necessary, including fencing, segregation and restriction of shared water or feed sources. At the regional level, cross-border data sharing and joint surveillance with neighboring regions in the Russian Far East should be strengthened to mitigate the risk of transboundary disease transmission. Given the porous nature of national borders for wildlife and the ecological continuity of habitats spanning China and Russia, the identified corridors are likely to represent transboundary viral dissemination pathways. In this context, early warning systems must transcend administrative boundaries and adopt integrated, binational frameworks that combine field surveillance with ecological modeling. Our findings highlight the need for bilateral coordination in surveillance timing, harmonization of diagnostic protocols, and data integration to capture wildlife movement and pathogen flow across landscapes. Therefore, this study provides not only a localized risk map but also a transferable approach that can guide joint monitoring, early outbreak detection, and coordinated mitigation strategies under a shared 'One Health' framework.

The four-stage workflow proposed in this study: habitat suitability modeling, risk overlap assessment, corridor identification and serological validation. It provides a practical and scalable framework for wildlife disease epidemiology and early warning systems. More broadly, this framework responds to a growing need for spatial tools that can inform 'One Health' approaches in regions where environmental change, wildlife movement and livestock expansion intersect. By identifying specific ecological corridors and high-risk zones at the human–wildlife interface, this study contributes to transdisciplinary strategies for anticipating and managing emerging zoonoses.

Conclusions: This study established a spatial framework integrating ecological niche modeling, risk mapping and serological validation to identify high-risk zones and potential transmission corridors of wild boar-borne viral diseases in eastern Heilongjiang, China. The results demonstrated a strong spatial overlap between suitable wild boar habitats and areas of elevated PRV and PCV2 seroprevalence, particularly in regions such as Hulin, Jixi, and Shuangyashan. Three major ecological corridors were identified, converging in Hulin, where hybrid wild boar farming coincides with high connectivity amplifying transmission risks. The framework presents targeted surveillance and cross-border early warning efforts, offering practical utility for regional wildlife disease preparedness under 'One Health' paradigm.

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