



Detection and prevalence of avian influenza epidemic in the southwest of Poyang Lake and analysis of the influence of meteorological factors

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ABSTRACT

Recently, the epidemiological profile of avian influenza has changed dramatically worldwide. Avian influenza sampling and surveillance of wholesale and retail markets in Nanchang, the largest city in the southwestern region of Poyang Lake, have been conducted since 2017. The transmission pattern of avian influenza in this region was comprehensively evaluated in multiple dimensions including time, subtype changes, seasonality and meteorological factors. Samples were tested for avian influenza A virus nucleic acids using real-time reverse transcription polymerase chain reaction, and positive results were typed. Wavelet coherence analysis was used to reveal the time-frequency variation in meteorological factors associated with avian influenza. The random forest algorithm was used to perform a multifactorial analysis of meteorological factors. Results revealed that the highest avian influenza positivity rate of 42.29 % (95 % CI: 41.18–43.41) occurred in summer. Meteorological factors were found to be significantly associated with the avian influenza positivity rate on a periodic basis. Random forest analysis revealed significant heterogeneity between meteorological factors and changes in the positivity rates of different avian influenza subtypes. Pollution concentration significantly affected the positivity rate of different avian influenza subtypes. The effect of temperature on the positivity rate of the H5 and H9 subtypes followed the opposite pattern to that of the non-H5/H7/H9 positivity rate. In winter, positivity rates of the H5 and H9 subtypes were lower and those of the non-H5/H7/H9 samples were higher; the opposite was true in spring. There is a correlation between pollutant concentration and avian influenza positivity rate. Authorities should consider climatic conditions and the level of contaminants in the prevention and control of avian influenza and adopt different preventive and control measures according to the characteristics of the different subtypes. We recommend continued surveillance of avian influenza in the region and the adoption of a 'one-health' approach for integrated prevention and control.

1. Introduction

Avian influenza is a global public health problem, and outbreaks of highly pathogenic avian influenza viruses have resulted in significant losses in the poultry industry and pose a serious threat to human health [1]. Starting in 1996, an outbreak of highly pathogenic avian influenza

caused by the H5 subtype was detected, which eventually led to a global epidemic [2]. In February 2013, the first human case of H7N9 avian influenza was reported in Shanghai, China, and it spread nationwide [3]. Between 2013 and 2017, China experienced five outbreak waves of H7N9 avian influenza, in which more than 1000 people were infected with the virus. Although the virus has not yet achieved human-to-human

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transmission, it has been transmitted from birds to humans. The mortality rate of patients infected with H7N9 is approximately 40 % [4]. The successive discovery and recurrent large-scale spread of highly pathogenic H5 and H7 avian influenza viruses in China is a problem that cannot be ignored. To address the avian influenza epidemic, the Chinese government implemented various countermeasures at an early stage. Since 2004, China has adopted a ‘culling + immunization’ prevention and control strategy to address the H5 avian influenza virus. This process is based on culling and isolation at outbreak sites, and mandatory vaccine immunization against avian influenza has been implemented for all poultry nationwide [5]. The Ministry of Agriculture has activated the ‘Recombinant Avian Influenza Virus (H5+H7) Bivalent Inactivated Vaccine (H5N1 Re-8 Strain + H7N9 H7-Re1 Strain)’ since the fall of 2017 and implemented comprehensive immunization of poultry nationwide [6].

Recently, with the continuous evolution of viruses, highly pathogenic avian influenza viruses have gradually broken the species bottleneck and spread among a wide range of mammals, which triggering panic [2]. The first report of highly pathogenic H5N1 avian influenza transmitted from wild birds to cattle and then to cattle workers through contact with sick cattle or the consumption of milk from sick cattle was reported in the United States in 2024, suggesting the importance of enhanced surveillance and control of the spread of avian influenza [7]. Migratory birds, especially waterfowl, are natural hosts of avian influenza, and along their migratory routes, they continuously reassort the avian influenza viruses they carry with birds in countries along the way, which may potentially lead to the emergence of novel avian influenza viruses and the cross-species and international spread of avian influenza [8]. Among the nine major migratory routes around the world, the East Asia-Australasia Migration Route is the largest, with millions of migratory birds flying each year from their breeding grounds in Siberia or Alaska to Australasia [9]. Poyang Lake is the largest freshwater lake in China and an important wintering and stopover site for migratory birds along the East Asia-Australia migratory route. Hundreds of thousands of migratory birds overwinter here every year; therefore, surveillance and research on avian influenza viruses in this region are crucial for preventing and controlling the international spread of avian influenza [6]. Studies have shown that there is seasonality in the spread of influenza viruses, and temperature and relative humidity are important factors affecting their spread of influenza viruses [10,11]. However, regional heterogeneity may exist in different areas. Avian influenza near Poyang Lake is affected by bird migration, and conditions such as temperature and humidity directly affect the food and water supply of birds, which may also impact the seasonality of avian influenza [12,13]. Therefore, it is necessary to analyze the drivers and factors affecting avian influenza in the region and elucidate the transmission pattern.

Wavelet coherence is a measure of the correlation between two time series in the frequency domain, which reveals the frequency domain correlation by calculating the degree of coherence between wavelet-transformed signals. Random forest is a machine learning method based on integrated learning that performs predictive analysis by constructing multiple decision trees. Each decision tree in a random forest is trained independently on a randomly selected subset, which can effectively reduce the risk of overfitting, and the results of decision trees are integrated to obtain the final prediction result [14]. Partial dependence analysis shows the marginal effect of a feature on the predicted outcome of a machine learning model, and partial dependence graphs show whether the relationship between the goal and the feature is linear, monotonic, or more complex. The individual conditional expectation is an extension of partial dependence that portrays the relationship between the predicted value of each individual and a single variable. The principle is as follows: for a certain individual and keeping other variables unchanged, randomly replace the values of selected feature variables and put them into the machine learning model to output the prediction results; finally, plot the relationship between a single feature variable and the predicted value for that individual [15]. Partial

dependence is the result of individual conditional expectations converging to the mean and reflecting the variable as a whole, whereas individual conditional expectations reflect the situation of each observed individual [16].

In this study, active surveillance of avian influenza virus in the southwestern region of Poyang Lake was conducted for approximately 8 years (2017–2024). We assess the transmission characteristics and epidemiological patterns of avian influenza virus in the region and analyze the heterogeneous transmission patterns of different subtypes of avian influenza virus. Additionally, the influence of meteorological factors on the spread of avian influenza is crucial. By collecting meteorological data in the region and using machine learning, mathematical, and other models, we can correlate those model with the surveillance data of avian influenza. Analyzing the change of avian influenza positivity rate with different meteorological conditions provides data support and theoretical basis for risk assessment and scientific prevention and control of avian influenza by relevant departments.

2. Materials and methods

2.1. Study design and avian influenza sample collection

Five cases of human H7N9 avian influenza outbreaks occurred in early 2017 in the Xinjian, Donghu, Xihu, and Qingshanhu Districts of Nanchang city (the southwestern region of Poyang Lake). Therefore, these four districts were chosen as long-term surveillance sites for avian influenza in wholesale and retail markets in Nanchang. Long-term surveillance of avian influenza was conducted from February 2017 to June 2024, with sampling of poultry in the market and the surrounding environment. Approximately 180 samples were collected per sampling event, of which poultry samples include oropharyngeal and cloacal swabs from chickens, ducks, pigeons, quails, geese, etc.; surrounding sampling includes, smear samples (countertops, frames, dirty buckets, etc.), wastewater (poultry wash water and poultry drinking water), and feces. For the remainder of the sampling period, the sampling process was conducted bimonthly with sampling occurring in even-numbered months. The sampling of avian influenza was divided into spring, summer, fall and winter according to the sampling season. As only nine samples of the H7 subtype (a small number) were collected during the surveillance period, this subtype was not analyzed in this study.

2.2. Laboratory testing and analysis

Sampling was carried out using commercial virus sampling tubes (with virus preservation solution) and disposable sterilized cotton swabs, which were collected and sent to the Nanchang Center for Disease Control and Prevention laboratory for storage within 48 h at 4 °C. Viral RNA was extracted from biological samples via a Liferiver Influenza Virus A real time RT-PCR kit (Liferiver, Shanghai, China) according to the manufacturer's instructions and identified via the M gene. Thereafter, the PCR-Fluorescence Probing Kit for Avian Influenza Virus Subtype (Daan, Gene, Guangzhou, China) was used to detect avian influenza virus H5, H7, and H9 subtypes in positive samples. Samples that are negative for H5, H7, and H9 subtypes but positive for avian influenza A virus are defined as non-H5/H7/H9 subtypes. Coinfection with multiple subtypes of avian influenza was not considered in this study. If coinfecting avian influenza samples were detected, they were counted separately for each subtype (e.g., if a sample was detected as infected with both H5 and H9 subtypes, the number of positive samples for each of the H5 and H9 subtypes increased by 1 regardless of H5 and H9 coinfection). In China, the isolation and cultivation of highly pathogenic avian influenza viruses require a Biosafety Level 3 (BSL-3) laboratory. Due to the limitations of our current laboratory conditions, this is currently difficult for us to achieve, which is one of the limitations of our study and has already been mentioned in the limitations section of the manuscript.

2.3. Meteorological data collection

In this study, local meteorological and air quality data from January 1, 2017 to May 30, 2024, in Nanchang city were collected, including the mean temperature (°C), maximum temperature (°C), minimum temperature (°C), mean air pressure (hPa), mean relative humidity (%), precipitation (mm), and mean wind speed (m/s). Air quality data included Carbon Monoxide (CO (mg/m³)) and Nitrogen Dioxide (NO₂) concentration (µg/m³). Meteorological data were obtained from the National Meteorological Science Data Center of China [17], and air quality data were obtained from the China Environmental Monitoring General Station [18].

2.4. Statistical analysis

2.4.1. Wavelet coherence analysis

Wavelet coherence analysis allows for the simultaneous analysis of the temporal and dimensional dependencies of two time series with high resolution and noise immunity. In addition, it not only accesses the local phase connectivity of time series in the time-frequency field but also provides significant correlation information [19]. The wavelet coherence coefficient between its time series is as follows:

$$R^2(s, \tau) = \frac{|S(s^{-1}W_{xy}(s, \tau))|^2}{S(s^{-1}|W_x(s, \tau)|^2) S(s^{-1}|W_y(s, \tau)|^2)}$$

where s is the scale, τ is the time shift, S is the smoothing factor, $W_x(s, \tau)$ and $W_y(s, \tau)$ are the wavelet transforms of the x and y sequences, respectively, and $W_{xy}(s, \tau)$ is the crossover wavelet spectra of the x and y sequences. In this study, wavelet coherence analysis was used to reveal the time-frequency associations between different avian influenza subtypes and meteorological factors. Monte Carlo randomization was used to assess whether the coherence of the time series was statistically significant. A wavelet coherence analysis graph was used to show the correlation between avian influenza positivity and meteorological factors. In the graph, the color represents wavelet coherence, with red indicating high coherence, and blue indicating low coherence. The horizontal axis represents the cumulative number of samplings over time and the vertical axis represents the wavelet oscillation period. The shaded portion of the plot represents regions where the calculated power spectrum is less accurate owing to the marginal effects. Coherent regions with statistically significant differences at the 5 % significance level are indicated by black lines. The arrows indicate the difference in phase of the time series. Upward arrows indicate that the avian influenza virus is leading in term of time or impact, and downward arrows indicate that meteorological factors are leading. Arrows to the left indicate that avian influenza viruses and meteorological factors are out of phase with each other and have countercyclical effects, whereas arrows to the right indicate that they are in phase with each other and have cyclical effects [20].

2.4.2. Random forest regression and partial dependence analysis

In this study, a random forest regression model was first used to fit the relationship between meteorological data and the positivity rates of different subtypes of avian influenza, and to determine the significance of each variable. Based on this model, partial dependence and individual conditional expectations were calculated to show the partial correlation between meteorological factors and the avian influenza positivity rate. R4.4.2 software was used for data processing, analysis, and visualization in this study, and $P < 0.05$ was statistically significant.

3. Results

3.1. Prevalence of different avian influenza subtypes

A total of 7570 samples were collected, of which 3035 were positive for at least one subtype of avian influenza virus, giving an overall positivity rate of 40.09 % (95 % CI: 38.99–41.20). Among these 3035 positive samples, the number of positive samples from avian species is 1646, and the number of positive environmental samples is 1389. For avian species, samples of oropharyngeal swabs and cloacal swabs were collected. The positive rates of oropharyngeal swabs and cloacal swabs for various subtypes of avian influenza in different species are shown in Table 1. The variation in month-to-month positivity rates for avian influenza was minimal, with the highest rate of 42.50 % (95 % CI: 41.39–43.61) in June and the lowest rate of 34.21 % (95 % CI: 33.14–35.28) in December (Fig. 1). The highest positivity rate for avian influenza subtype H5 was 3.73 % (95 % CI: 3.30–4.16), which was observed in August, while the lowest was observed in December (0.56 % (95 % CI: 0.39–0.72)). The highest positivity rate for avian influenza subtype H9 was 33.49 % (95 % CI: 32.43–34.56), which was observed in August, while the lowest was observed in December (16.19 % (15.36–17.02)). The highest positivity rate for avian influenza non-H5/H7/H9 subtypes were 17.70 % (95 % CI: 16.84–18.56), which was observed in December, while the lowest was observed in April (7.46 % (95 % CI: 6.87–8.05)).

3.2. Prevalence of avian influenza subtypes in different seasons

During the surveillance period, samples positive for avian influenza were collected throughout the year; however, there were variations in the rate of positivity for different subtypes of avian influenza in different seasons (Fig. 2). The overall avian influenza positivity rate was highest in summer at 42.29 % (95 % CI: 41.18–43.41) and lowest in winter at 36.69 % (95 % CI: 35.61–37.78). For the H5 subtype, the highest positivity rate was 2.86 % (95 % CI: 2.48–3.23) in spring and the lowest positivity rate was 1.17 % (95 % CI: 0.93–1.41) in winter. The positivity rate of the H9 subtype was the highest in spring, accounting for 32.46 % (95 % CI: 31.41–33.52), and the lowest in winter, accounting for 19.37 % (95 % CI: 18.48–20.26). For non-H5/H7/H9 subtype samples, the positivity rate for avian influenza was highest in the winter at 16.57 % (95 % CI: 15.73–17.41) and lowest in the spring at 7.46 % (95 % CI: 6.87–8.05).

3.3. Wavelet coherence analysis of avian influenza meteorological factors

Meteorological data collected each day were matched with data from the day of avian influenza sampling to obtain the meteorological dataset at the time of each sampling, and the variables were statistically described and analyzed for correlation (Supplementary Fig. 1). Since the correlation coefficients between the mean temperature, maximum temperature, minimum temperature, and mean pressure were all greater than 0.6, there was a high degree of correlation; therefore, the mean temperature was ultimately chosen to replace these variables in the analysis. Wavelet coherence analysis was then used to determine the relationships between overall avian influenza positivity, H5 subtypes, H9 subtypes, non-H5/H7/H9 avian influenza positivity, and different meteorological factors (Fig. 3; Supplementary Figs. 2–4). The results revealed that the mean temperature, precipitation, mean relative humidity, mean wind speed, and CO and NO₂ concentrations were highly correlated with the different subtypes of avian influenza in terms of period. Climatic factors were also found to lead to changes in avian influenza positivity rates, suggesting that changes in climatic factors lead to changes in the avian influenza positivity rate.

Table 1
The positive rates of oropharyngeal swabs and cloacal swabs for various subtypes of avian influenza in different species.

	Overall positivity rate (%)		H5 positivity rate (%)		H7 positivity rate (%)		H9 positivity rate (%)		Non-H5/H7/H9 subtypes positivity rate (%)	
	Oropharyngeal swab	Cloacal swab	Oropharyngeal swab	Cloacal swab	Oropharyngeal swab	Cloacal swab	Oropharyngeal swab	Cloacal swab	Oropharyngeal swab	Cloacal swab
Chicken	978(58.42)	75 (27.99)	17(1.02)	0(0.00)	0(0.00)	0(0.00)	897(53.58)	66 (24.63)	79(4.72)	9(3.36)
Duck	519(33.14)	55 (17.86)	39(2.49)	6(1.95)	1(0.06)	0(0.00)	201(12.84)	23(7.47)	296(18.90)	28(9.09)
Goose	4(40.00)	0(0.00)	1(10.00)	0(0.00)	0(0.00)	0(0.00)	1(10.00)	0(0.00)	3(30.00)	0(0.00)
Pigeon	15(19.23)	0(0.00)	2(2.56)	0(0.00)	0(0.00)	0(0.00)	8(10.26)	0(0.00)	5(6.41)	0(0.00)
Quail	0(0.00)	–	0(0.00)	–	0(0.00)	–	0(0.00)	–	0(0.00)	–
Environment	1389 (37.98)		98 (2.68)		8 (0.22)		866 (23.68)		478 (13.07)	

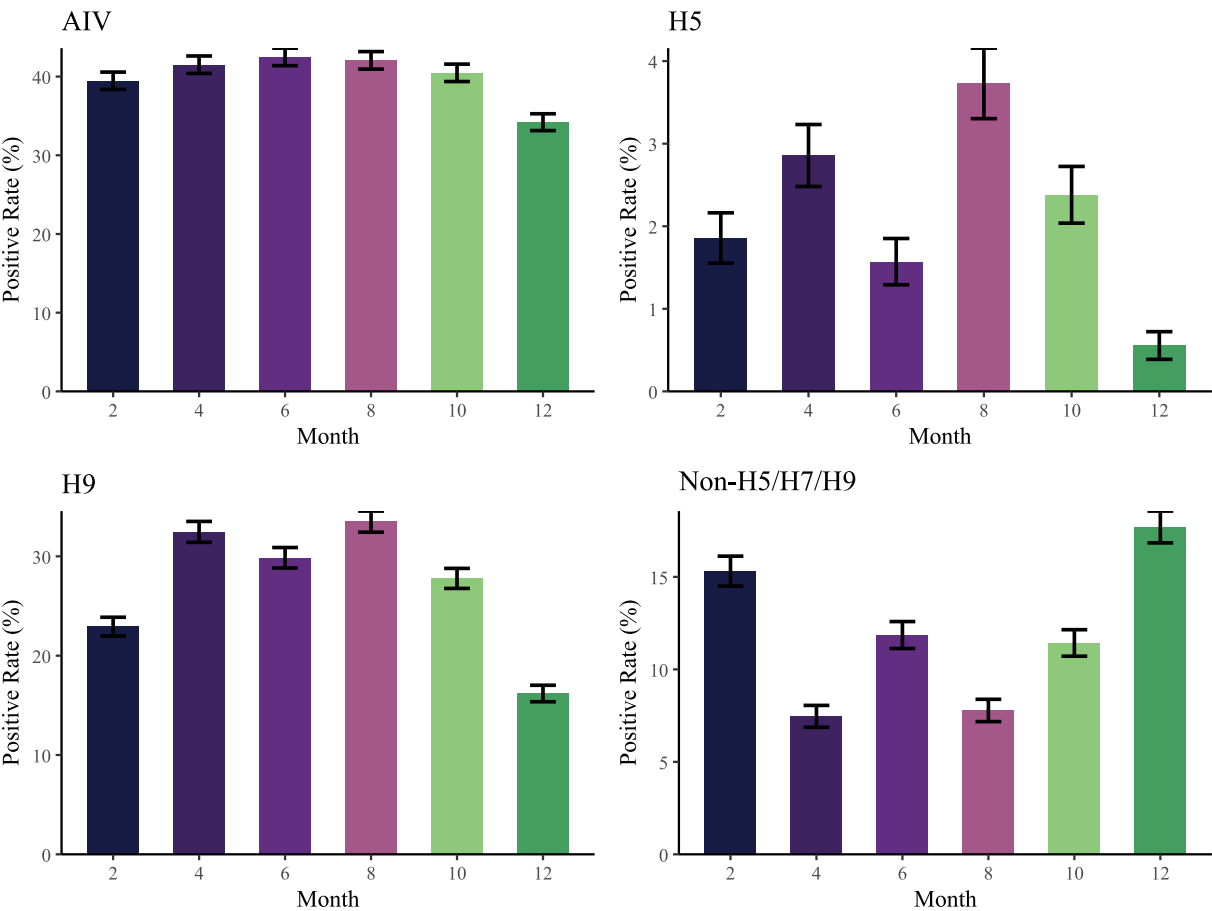


Fig. 1. The positivity rate in even months for different avian influenza subtypes form 2017–2024 (AIV: overall avian influenza positivity rate; H5: avian influenza positivity rate for the highly pathogenic H5 subtype; H9: avian influenza positivity rate for the H9 subtype; Non-H5/H7/H9: avian influenza positivity rate for the non-H5/H7/H9 samples).

3.4. Analysis of meteorological factors via random forests and partial dependence

IncNodePurity was used to assess the importance of the variables in the random forest model, with larger values indicating that the variables are more important and have a greater impact on the results. The results revealed differences in the important variables affected by different subtypes (Fig. 4). For the overall avian influenza, CO, NO₂, and mean wind speed were the three most influential variables; for the H5 avian influenza subtype, mean relative humidity, CO, and NO₂ were the three most influential variables; for the H9 avian influenza subtype, NO₂, CO, and mean temperature were the three most influential variables; and for the non-H5/H7/H9 samples, NO₂, mean wind speed, and mean

temperature were the three most influential variables. The variable importance in the random forest can only indicate the magnitude of the influence of features on model prediction. Furthermore, we plotted partial dependence plots and individual conditional expectation curves to show the changes in the positivity rate of avian influenza under different conditional variations (Fig. 5; Supplementary Figs. 5–7). The overall positivity rate of avian influenza peaked at 11 °C and was the relatively low at approximately 18 °C. The positivity rate of avian influenza was highest when the mean relative humidity was approximately 70 %. With increasing precipitation, the overall positivity rate for avian influenza tended to decrease. For the H5 subtype, when the mean temperature was higher than 30 °C, the positivity rate increased significantly. When the mean relative humidity was either lower than

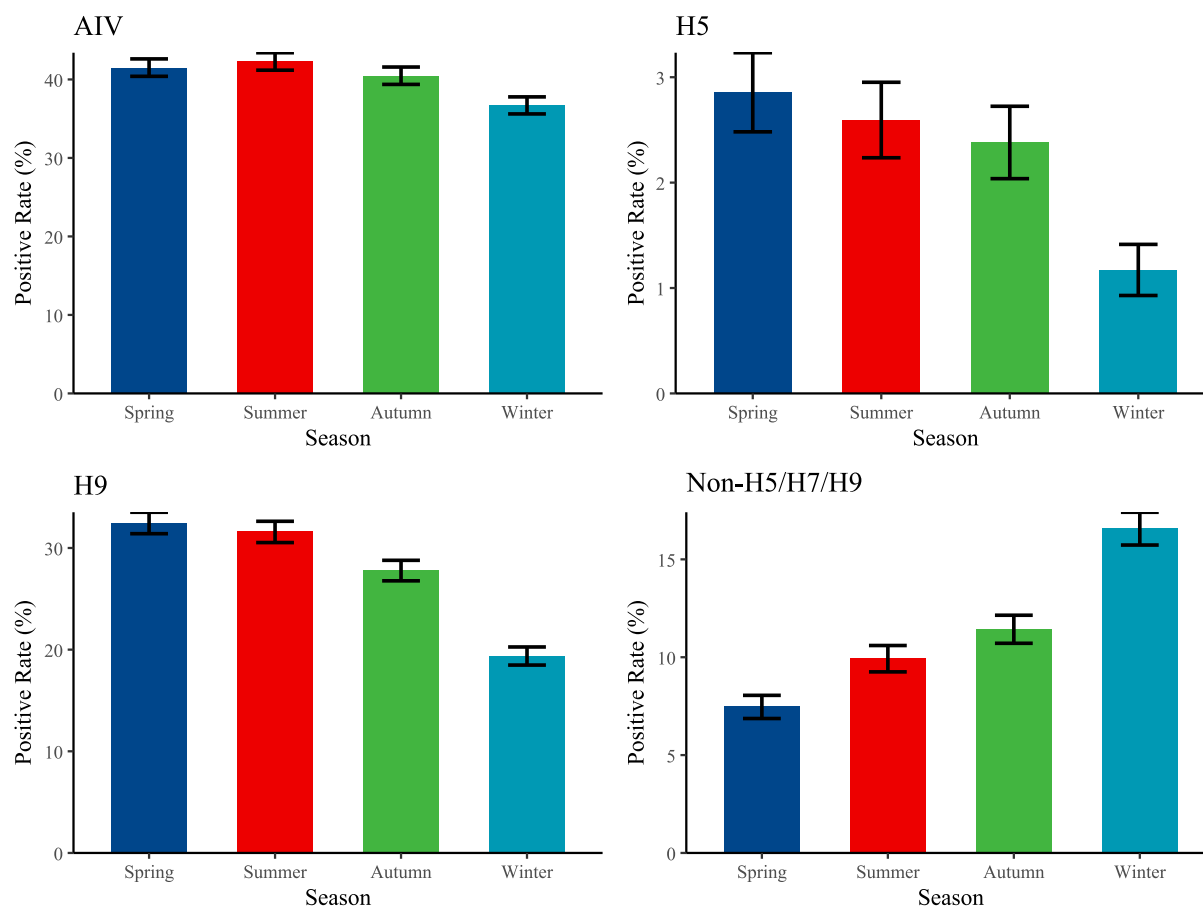


Fig. 2. Seasonal positivity rates for different avian influenza subtypes from 2017 to 2024 (AIV: overall avian influenza positivity rate; H5: avian influenza positivity rate for the highly pathogenic H5 subtype; H9: avian influenza positivity rate for the H9 avian influenza subtype; Non-H5/H7/H9: avian influenza positivity rate for the non-H5/H7/H9 samples).

50 % or higher than 60 %, the positivity rate of the H5 subtype increased remarkably. Precipitation had little effect on the positivity rate for the H5 subtype. For the H9 subtype, the effect of temperature on this subtype showed a monotonically increasing trend; with increasing temperature, the positivity rate gradually increased, and when the temperature was near 20 °C, the growth rate was obvious; when the mean relative humidity was in the vicinity of 68 %, the positivity rate was the highest; precipitation in the 15 mm later, with the increasing in precipitation its positivity rate gradually decreased. For the non-H5/H7/H9 samples, the effect of temperature showed a unimodal distribution, with the highest positivity near 10 °C which then decreased with increasing temperature. Mean relative humidity and precipitation had little effect on the positivity of this subtype. Higher wind speed and pollutant concentrations (CO and NO₂) significantly reduced the H5 and H9 subtypes of avian influenza. However, the effects of the mean wind speed and NO₂ concentration on overall avian influenza positivity showed an inverted U shape, with the lowest overall avian influenza positivity at a mean wind speed of 2.5 m/s. Subsequently, the positivity rate began to increase as the wind speed increased. The overall avian influenza positivity rate was lowest at NO₂ concentrations near 38 µg/m³, and its positivity rate gradually increased with increasing NO₂ concentration. The non-H5/H7/H9 positivity rate decreased with increasing mean wind speed and CO concentration but increased with increasing NO₂ concentration.

4. Discussion

In this study, avian influenza in Nanchang city, in the southwest region of Poyang Lake, was surveyed for 8 years. Comprehensive

analyses were conducted in terms of time, subtype changes, seasonal effects, and air quality-related factors, contributing to the transmission pattern of avian influenza in the region. The association of seasonal effects and meteorological factors with the spread of avian influenza in the migratory region of migratory birds was explored and served as the basis for recommendations to mitigate the spread of avian influenza viruses in the region in the future. China is a major poultry farming country with a high preference for live poultry. Three of the world's nine major migratory routes pass through China, making it a country with a high risk of infection with subtypes of avian influenza and the emergence of new subtypes with the potential to infect humans. Climate change can accelerate ecosystem shifts and thus alter the evolution and ecology of infectious diseases, especially avian influenza viruses. Migratory bird migration increases the rate of spread of avian influenza and the risk of international transmission [21]. The overall positive rate of avian influenza in the monitored area was much higher than that in China (12.73 %), indicating a relatively high prevalence of avian influenza in the southwestern area of Poyang Lake [22]. Further comparison with other regions belonging to the same Poyang Lake region revealed that the positive rate of avian influenza in Shangrao city in the Poyang Lake region even reached 60.50 %, which indicates that avian influenza in the Poyang Lake region may have its own heterogeneity [6]. Highly pathogenic avian influenza, with its high rates of transmission and mortality, has long been a source of concern. Recent outbreaks of genetic variants and mutations of the H5N1 strain in wild birds, poultry, and cattle have raised serious concerns about potential transmission and public health risks [23]. Therefore, studying avian influenza in this region is important for public health.

Avian influenza is considered a seasonal infection, and analysis of

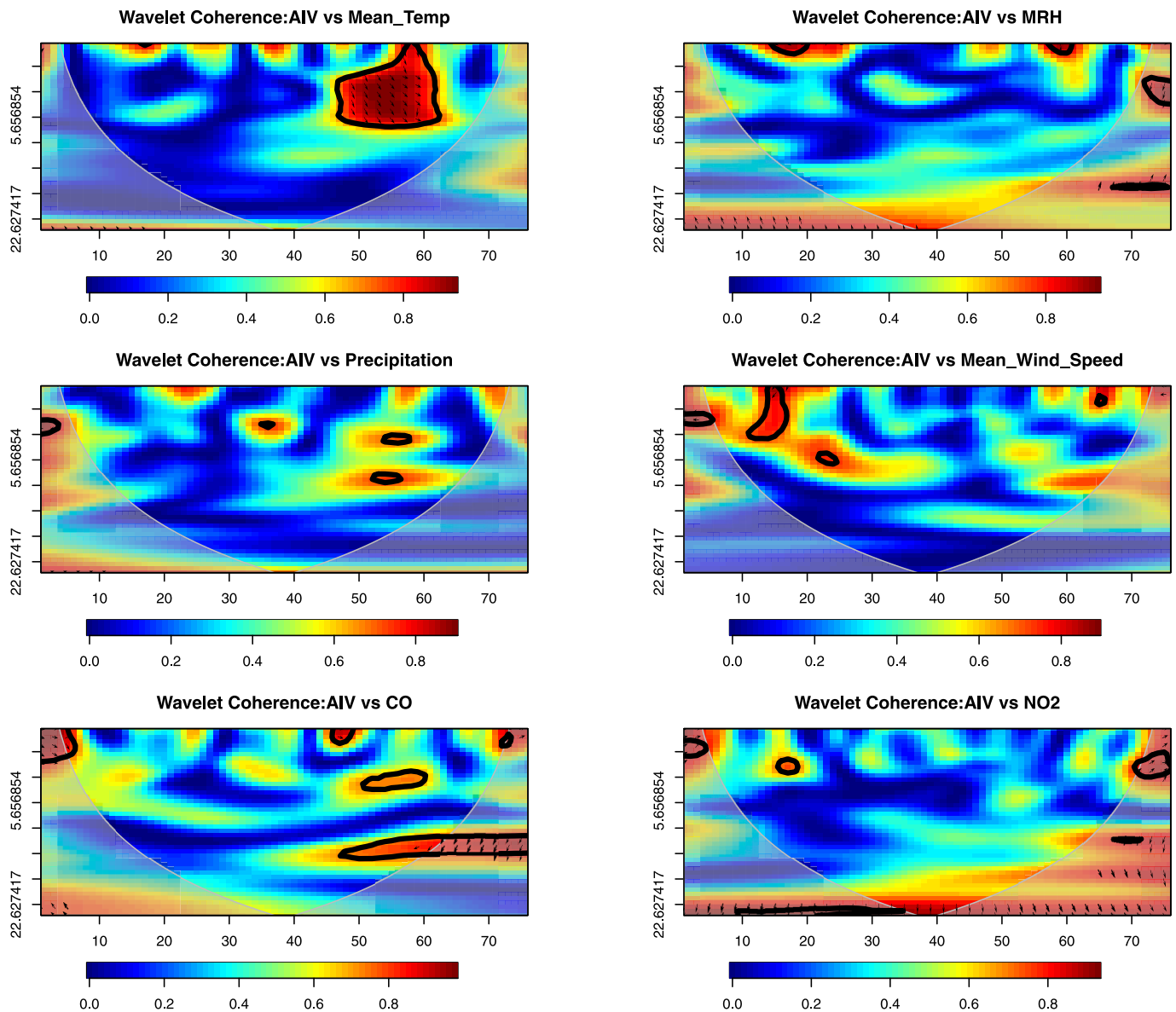


Fig. 3. Wavelet coherence analysis of overall avian influenza positivity with different meteorological factors (Mean_Temp: Mean temperature ($^{\circ}\text{C}$); MRH: Mean relative humidity (%); Precipitation: Precipitation (mm); Mean_Wind_Speed: Mean wind speed (m/s); CO: CO concentration (mg/m^3); NO_2 : NO_2 concentration ($\mu\text{g}/\text{m}^3$). The color spectrum indicates wavelet coherence, with red indicating strong coherence and blue indicating weak coherence. It is a function of the study period (x-axis) and the oscillatory period (y-axis). The black line indicates the coherence region at the 5% significance level, and the shaded portion indicates the less accurate part of the power spectrum calculated due to boundary effects). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

avian influenza in most countries has shown that outbreaks are more likely to occur during cooler seasons. However, this study obtained anomalous results from 8 years of surveillance in the southwestern region of Poyang Lake [24]. We found that in this region the positivity rate for H5 and H9 subtypes of avian influenza is instead lower in winter and higher in spring and summer, but the opposite occurs for non-H5/H7/H9 avian influenza, which is higher in winter and relatively lower in spring and summer. According to the surveillance of avian influenza in the external environment in Fujian Province from 2017 to 2021, the positivity rate was higher in winter, spring and summer, which is similar to our results but not completely due to differences in geography and the type of sampling between the two [25]. In addition, our analysis of meteorological factors revealed that the H5 and H9 subtype positivity rates gradually increased with increasing temperature, whereas the non-H5/H7/H9 positivity rate gradually decreased with increasing temperature, which is consistent with our observations. Certain studies have

demonstrated a significant correlation between migratory birds and changes in airborne avian influenza positivity [26], and our study area is located in the largest migratory base of migratory birds in China. Different types of birds have varying degrees of adaptation to climatic conditions. In the Poyang Lake area, wintering migratory birds spend the winter from October to April of the following year, whereas summer migratory birds leave the lake from the end of August to the beginning of October. The alternation of migratory bird migrations is highly seasonal, which may be the reason for the differences in the positivity rates of avian influenza in this region compared with those in other regions and the significant differences in different subtypes of avian influenza viruses in this area in different seasons. However, continuous monitoring and in-depth research are required [27].

Many studies have investigated the spread of the virus and discussed possible risk factors; however, climate change factors have been largely neglected. Changes in the avian influenza positivity rate are closely

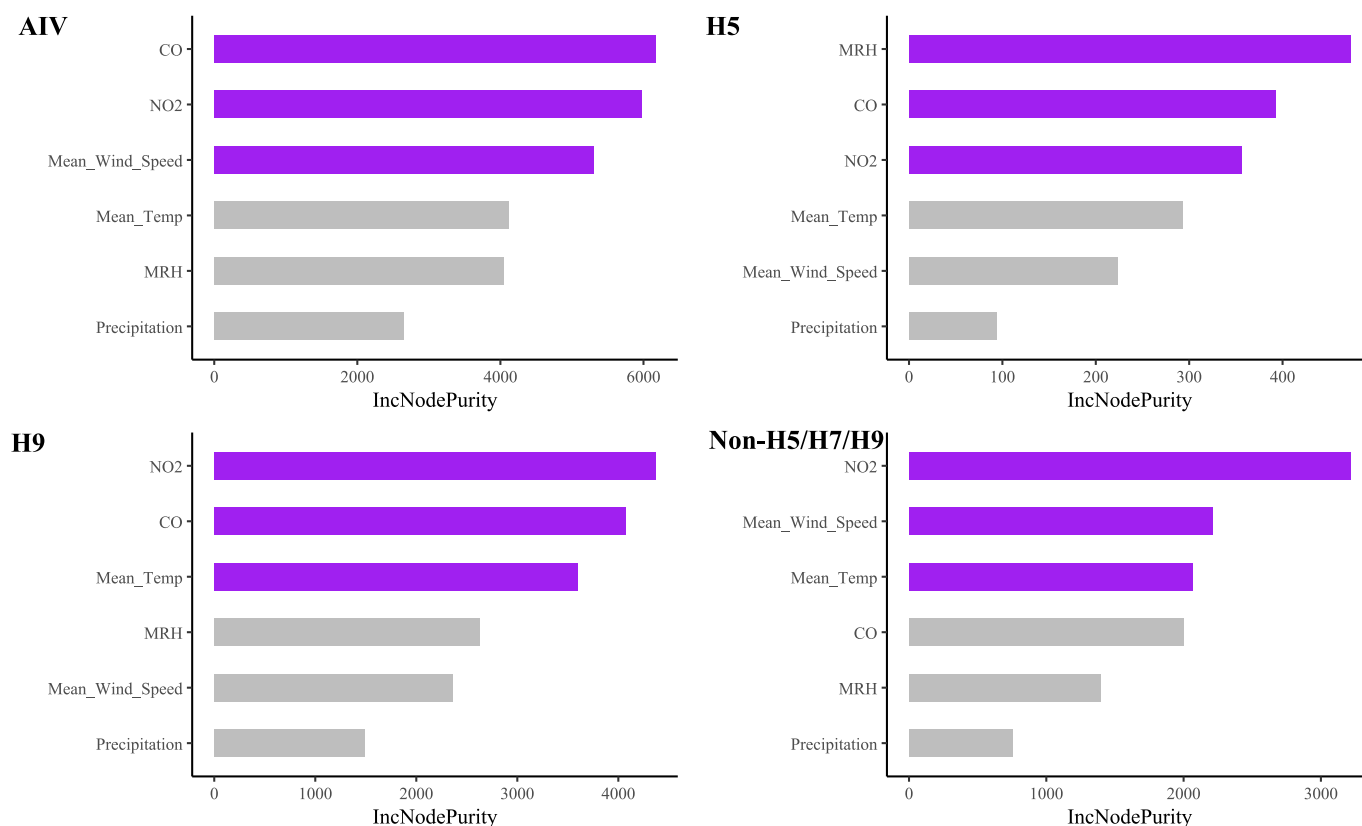


Fig. 4. Random forest model variable importance analysis (AIV: overall avian influenza positivity rate; H5: highly pathogenic H5 subtype avian influenza positivity rate; H9: H9 subtype avian influenza positivity rate; Non-H5/H7/H9: avian influenza positivity rate in non-H5/H7/H9 samples. IncNodePurity, i.e., an increase in node purity, is measured by the sum of squared residuals. It represents the effect of each variable on the heterogeneity of observations at each node of the classification tree. Thus, it compares the importance of variables, with larger values indicating greater importance of the variable).

related to changes in climate, which alters the viability of the avian influenza virus and leads to a change in its duration [28]. Most meteorological studies on avian influenza have analyzed only temperature and humidity, but air quality and other aspects have been neglected. Our findings show that not only meteorological factors but also air pollution-related factors affect the positivity rate of avian influenza, and that the factors that are affected vary among subtypes. The effects of meteorological and air quality factors on avian influenza are nonlinear, with air quality factors (CO and NO₂) showing the greatest effect on various subtypes of avian influenza, which has rarely been mentioned in previous studies. In China, industrial emissions are an important source of atmospheric pollutants [29]. Many industrial products generate CO and NO₂. In addition, vehicle exhaust is also an important source of CO and NO₂. Most agricultural machinery such as tractors and harvesters that are widely used on farms, are fueled by diesel. These machines emit CO and NO₂ during operation, thereby causing pollution. In some regions, there is the phenomenon of straw burning. When straw is incompletely burned, CO is generated, and the high temperature during the burning process also promotes the generation of NO₂. The smoke generated by straw burning spreads across the farm and its surrounding areas, resulting in an increase in the concentrations of CO and NO₂ in the air [30]. When there are industrial facilities such as factories and power plants around the farm, the CO and NO₂ they emit will diffuse into the farm area along with atmospheric circulation, thus increasing the concentration of pollutants on the farm. These are all issues that deserve attention. In the Poyang Lake area, temperature, humidity, wind speed affect the positivity rate of various avian influenza subtypes, but a certain critical value exists, and the positivity rate increases or decreases greatly after reaching this critical value. Notably, the effect of NO₂ on the positivity rate of H5 and H9 subtypes of avian influenza was opposite to that of non-H5/H7/H9 avian influenza, with the former showing a

substantial decrease after reaching the threshold and the latter a substantial increase. This problem was also observed in the analysis of avian influenza subtypes in different seasons, where the variation in the positivity rate of non-H5/H7/H9 avian influenza was opposite to that of the H5 and H9 subtypes, which may be due to differences in the biological characteristics of viruses and their ability to interact with their hosts, which leads to differences in the susceptibility of their different subtypes to different climatic conditions [31]. Although few studies have shown a risk relationship between NO₂ and avian influenza, a Chongqing influenza study revealed that there is a plausible causal mechanism between NO₂ and influenza infection and that it may indirectly affect influenza by influencing other factors. Therefore, we suspect that the same mechanism may exist for avian influenza, which warrants further investigation [32]. Our study revealed the potential effects of influencing factors on avian influenza at different critical points and quantified them via lines. These findings provide an important basis for relevant authorities to formulate scientific, precise prevention and control strategies for avian influenza.

This study also has a number of limitations. First, due to insufficient laboratory facilities, we performed real time RT-PCR for highly pathogenic avian influenza, but did not culture, isolate or genetically sequence the influenza positive samples reported in the present study; hence, the genetic characterization of these positive sample could not be established. In addition, in the analysis of meteorological factors, data were missing for the month of June, which may have affected the comprehensiveness of the analysis to some extent. Finally, there may be other factors associated with the positivity rate of avian influenza that were not included in the analysis of this study.

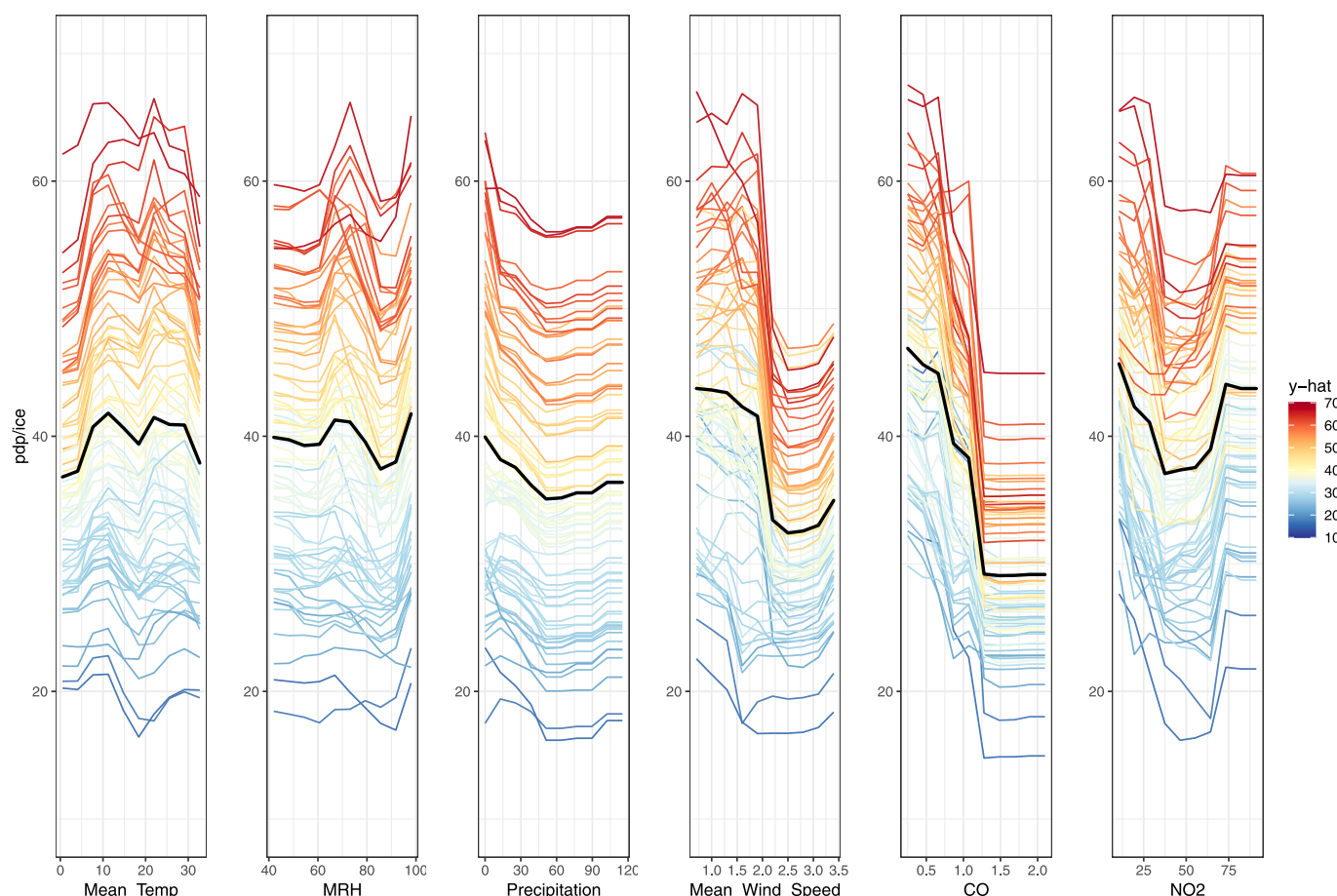


Fig. 5. A partial dependence plot of meteorological factors on the overall avian influenza positivity rate (Mean_Temp: Mean temperature ($^{\circ}\text{C}$); MRH: Mean relative humidity (%); Precipitation: Precipitation (mm); Mean_Wind_Speed: Mean wind speed (m/s); CO: CO concentration (mg/m^3); NO₂: NO₂ concentration ($\mu\text{g}/\text{m}^3$). The black line is the partially dependent value which is the mean value of the individual conditional expectation, and the other lines represent the conditional expectation of each individual).

5. Conclusion

Long-term surveillance and analysis revealed that the positivity rate for avian influenza in the southwestern region of Poyang Lake was relatively high. The positivity rates for the H5 and H9 subtypes were lower in winter but higher in spring, while the opposite was true for the non-H5/H7/H9 subtypes. Analysis of meteorological conditions and pollutant concentrations revealed that the positivity rates of different subtypes of avian influenza were affected differently by temperature, CO, and NO₂ concentrations. Based on these findings, precise prevention and control strategies should be formulated by combining the climate conditions and pollutant levels. Be vigilant against the transmission risks of H5 and H9 subtypes during the summer season and strengthen the management and control of non-H5/H7/H9 samples during the winter period. Moreover, public health education should be strengthened to increase awareness and protection capabilities of practitioners and the public regarding the transmission patterns of avian influenza.

CRedit authorship contribution statement

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Ethical approval statement

The study and the analysis plan were approved by the Ethics Committee of Nanchang Center for Disease Control and Prevention (NCCDC 2016–006).

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.onehlt.2025.101047>.

Data availability

Data will be made available on request.

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