Spatio-temporal epidemiology of Japanese encephalitis virus infection in pig populations of eastern Uttar Pradesh, India, 2013-2022

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Running Head: Spatio-temporal epidemiology of JE in pig populations in India

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Summary

Japanese encephalitis (JE) is endemic in India. Although pigs are considered an important amplifier host as well as sentinels for JE outbreaks in people, limited information is available on JE virus (JEV) surveillance in pigs. We investigated the spatio-temporal distribution of JEV seroprevalence and its association with climate variables in 4451 pigs sampled from 10 districts in eastern Uttar Pradesh, India over a 10 year period (2013—2022).

The mean seroprevalence of IgG (2013-2022) and IgM (2017—2022) was 14% (95% CI 12.8—15.2) and 10.98% (95% CI 9.8—12.2), respectively. Throughout the region, higher seroprevalence from 2013—2017 was observed, and was highly variable with no predictable spatio-temporal pattern between districts. Seroprevalence of up to 60.8% in Sant Kabir Nagar in 2016 and 69.5% in Gorakhpur district in 2017 for IgG and IgM was observed, respectively. IgG seroprevalence did not increase with age. Monthly time series decomposition of IgG and IgM seroprevalence demonstrated annual cyclicity (3-4 peaks) with seasonality (higher, broader peaks in the summer and monsoon periods). However, most variance was due to the overall trend and the random components of the time series. Autoregressive time-series modelling of pigs sampled from Gorakhpur was insufficiently predictive for forecasting; however, an inverse association between humidity (but not rainfall or temperature) was observed.

The results confirm year-round endemicity in pigs in eastern Uttar Pradesh. Transient IgG suggests that JEV might not be immunising in pigs, which needs further investigation; some models that inform public health interventions for JEV assume long-term immunity in pigs. Although pigs are used as sentinels for human outbreaks, we find that most variability in pig seroprevalence is not due to underlying trend or annual cyclicity. Therefore, autoregressive models using broad climate variables are most likely insufficiently nuanced to inform public health interventions for JEV prevention in people.

Keywords: time series; Japanese encephalitis virus; India; spatial epidemiology; swine

Impacts

- Study findings suggest that JEV might not be an immunising infection in pigs. This has important implications where modelling studies assuming long-term immunity are used to support public health interventions. Longitudinal studies of pig immunity following natural JEV infection are needed to test this hypothesis.
- JEV transmission in pigs occurs year-round in eastern Uttar Pradesh, India, demonstrating potential for transmission to people if competent mosquito vectors are present.
- Detailed, local environmental data are likely required to develop models useful for public health surveillance in this endemic region, where pig sero-surveillance is currently more likely to be useful to confirm, rather than predict district-wise high risk periods and the need to intensify public health resources to prevent JEV among people in a district.

Introduction

Japanese encephalitis (JE) is a mosquito-borne flaviviral zoonosis that causes encephalitis in people in the Asia-Pacific region (Wang & Liang, 2015). It is estimated that >1 billion people live in areas suitable for local JE virus (JEV) transmission (Moore, 2021) and annually, JEV has been estimated to cause 68,000 human cases, including 13,600-20,400 deaths, with estimated 51,000 (75%) cases occurring in children [?]14 years old (Campbell et al., 2011). Although most JEV infections are mild (fever and headache) or asymptomatic, approximately 1 in 250 infections results in severe clinical illness (Amicizia, Zangrillo, Lai, Iovine, & Panatto, 2018). The case fatality rate can reach 30%, and of those who survive, 30% suffer permanent neurological sequelae including 'locked-in syndrome' (World Health Organization, 2019).

Japanese encephalitis virus is a multi-host pathogen and reservoir species include ardeid birds and pigs, with mosquitoes (primarily *Culex* sp.) as biological vectors (Wang & Liang, 2015). People are incidental, non-competent hosts, also becoming infected via the bite of *Culex* species mosquitoes. There is no specific antiviral treatment for patients affected with JEV; therefore, supportive medication is given to relieve symptoms and stabilize the patient. In India, the incidence and case fatality rate of acute encephalitis syndrome (AES) – in which JE is identified in 5-20% of AES cases – has fallen since 1978 through implementation of JE vaccination campaigns, improved surveillance, and more rapid treatment at designated Encephalitis Treatment Centres (Srivastava, Deval, Mittal, Kant, & Bondre, 2021).

Japanese encephalitis has been recognised in several tropical and subtropical states of India, and recent JEV spread in the northern temperate states of India has been reported (Mote et al., 2023). In eastern Uttar Pradesh (UP) state (Kumari & Joshi, 2012; A. K. Singh et al., 2020; G. Singh et al., 2013), extensive and recurrent outbreaks are reported, and together with the state of Assam, contribute the greatest proportion of the total JE burden in India; from 2015-2021, 11,326 human JE cases were reported from 23 states in India, with 2265 and 3334 cases (49% overall) from the states of Assam and Uttar Pradesh, respectively (Directorate of National Vector Borne Disease Control Programme, 2022).

Multiple landscape factors including topography, waterlogged rice-paddy fields, piggeries without mosquito control, pig-keeping in residential areas, high pig populations, and the prolonged rainy season support the breeding of *Culex* mosquitoes and perpetuate the transmission of JEV to pigs people in these two states (Khan et al., 1996; Kumari & Joshi, 2012; Murhekar, Vivian Thangaraj, Mittal, & Gupta, 2018). Rainfall has been demonstrated to be strongly associated with JEV outbreaks in India (Borah, Dutta, Khan, & Mahanta, 2013; H. Singh, Singh, & Mall, 2020), and recent analysis of India-wide JEV incidence in people indicated that outbreak risk was strongly associated with the habitat suitability of ardeid birds, both pig and chicken densities, and the shared landscapes between fragmented rain-fed agriculture and both river and freshwater marsh wetlands (Walsh et al., 2022).

In endemic regions, JEV can circulate in pig populations because their high birth rate provides a continuous supply of susceptible pigs and their viraemia levels are sufficient to infect mosquitoes for 3-5 days – hence they are known as amplifying hosts (Ladreyt, Durand, Dussart, & Chevalier, 2019). In Asia, pigs are preferentially fed on by the main mosquito vector of JEV between pigs and people, *Culex tritaeniorhynchus* , and pigs have been shown to seroconvert 2-3 weeks before JEV infection is detected in humans (Borah et al., 2013; Cappelle et al., 2016; G. Singh et al., 2013; Van den Hurk, Ritchie, & Mackenzie, 2009). Sero-surveillance of pig populations can therefore be an important component of JEV surveillance to estimate the risk of zoonotic transmission and guide prevention strategies including mosquito control, bite prevention, and human or pig vaccination programs (Duong et al., 2011; Kumar et al., 2020; Ruget et al., 2018). Despite this, few JEV studies have investigated the use of sero-surveillance of JEV in pigs. In a study of IgG and IgM seroprevalence for JEV in 488 pigs in endemic regions in India (Kolhe et al., 2015), IgG ranged from 20% in Uttar Pradesh to 35.2% in Northeast India. In contrast, IgM seroprevalence in the same samples was highest in Goa (39%) and lowest in Northeast India (22.7%) and Uttar Pradesh (20%). Overall, higher seroprevalence of IgM was detected, possibly because the study was conducted in June to October when increased transmission of JEV occurs and pigs were recently infected. In an earlier study of IgG seroprevalence in 500 pigs from April 2013 – May 2014, seroprevalence was 61.5% in Tripura, 29.3% in Uttar Pradesh, and 28.7% in Punjab, with highest overall seroprevalence between July and October (H. Dhanze et al., 2014). Whilst these studies give some insight about JEV epidemiology in pigs – for example, the relative seroprevalence in pigs between regions and months – there is limited detail about trend, associations with climate or landscape variables, and differences between IgG and IgM antibodies.

The objective of this study was to describe the spatio-temporal distribution of JEV seroprevalence in the pig population of eastern Uttar Pradesh, India between 2013 and 2022, and investigate seasonality, trend, and associations between seroprevalence and climate variables (rainfall, temperature, and humidity) in the region. The aim of this study was to provide insights about the epidemiology of JEV in pigs in the region over a longer period than has previously been conducted and assess how sero-surveillance of JEV in pigs could be most useful to inform disease control programs to prevent JEV impact in people.

Materials and Methods

Study area and data collection

The study was conducted in the state of Uttar Pradesh (UP), where there are 18 administrative divisions divided into a total of 75 districts (https://www.up.gov.in, accessed 3 May 2023). The state of UP has 3 distinct seasons: winter from November to February, summer from March to May, and monsoon from June to October (https://www.nri.up.gov.in, accessed 3 May 2023). Five districts in eastern UP in Gorakhpur and Basti divisions (Gorakhpur, Maharajganj, Kushinagar, Sant Kabir Nagar and Siddarthnagar districts) are frequently affected by floods due to the topography of the region(Kumari & Joshi, 2012). During 2011-2018, the four districts of Gorakhpur division (Gorakhpur, Maharajganj, Deoria and Kushinagar), and the three districts of Basti division (Basti, Sant Kabir Nagar and Siddarthnagar) contributed to 86% of the total number of reported acute encephalitis syndrome cases in UP (A. K. Singh et al., 2020).

Pig serum samples were collected from three divisions of eastern UP: Gorakhpur (Gorakhpur, Kushinagar, Deoria and Maharajganj districts), Basti (Basti, Sant Kabir Nagar, Siddarthnagar districts) and Azamgarh (Azamgarh, Ballia, and Mau districts; Figure 1). The samples from Gorakhpur and Basti divisions were collected from February 2013 to December 2022, whereas samples from Azamgarh division were collected from 2017 to 2022, following increased reporting of Japanese encephalitis (JE) cases in people in Azamgarh division. Samples were collected by veterinarians of the State Animal Husbandry Department and Indian Veterinary Research Institute and submitted to the Division of Veterinary Public Health, Indian Veterinary Research Institute, Bareilly, UP, for sero-diagnosis of JE. Variables recorded at the time of sample collection included pig age (categorised into 3 groups: 4-5 months, 6-12 months and >12 months), date of sample collection, and district from where the sample was collected.

Monthly total rainfall, mean relative humidity and mean minimum temperature measured at Gorakhpur Station (latitude 26.74, longitude 83.45) during the study period were obtained from 'The Visual Crossing global weather database' (https://www.visualcrossing.com/weather-data, accessed 5 May 2023).

Animal ethics approval was not required for this study, because collection of samples and analysis are conducted for pre-existing and ongoing surveillance programmes for animal and public health.

Sample processing

Pig samples were separated, and serum analysed for the presence of antibodies against JEV using indirect IgG ELISA (H Dhanze et al., 2019) and indirect IgM ELISA (H. Dhanze, Bhilegaonkar, et al., 2020). The diagnostic sensitivity and specificity of the indirect IgG ELISA was 91% and 97%, respectively, while the diagnostic sensitivity and specificity of the indirect IgM ELISA was 95.34% and 98.6%, respectively. The IgM ELISA for sero-diagnosis of JEV was developed and validated in 2017 and therefore, samples prior to September 2017 were screened only for the presence of IgG antibodies against JEV.

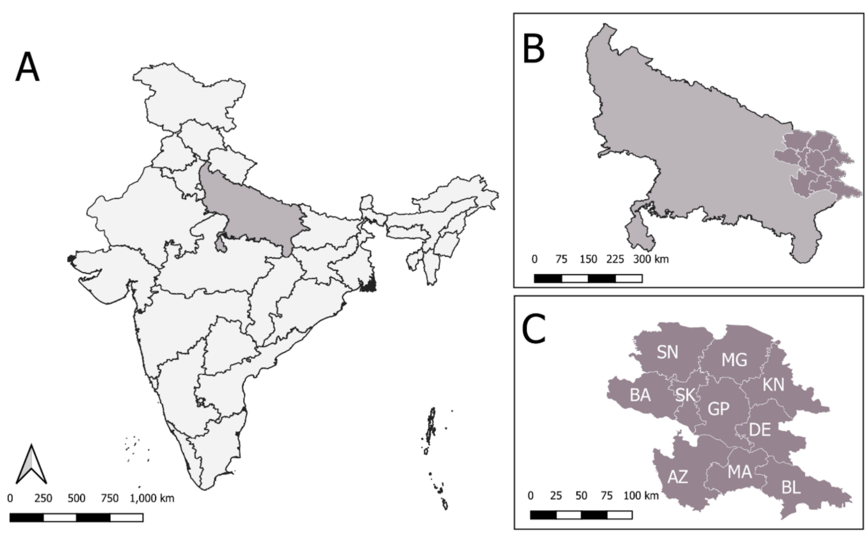

Figure 1 Study area of Japanese encephalitis virus seroprevalence in the pigs. A: India highlighting Uttar Pradesh; B: Uttar Pradesh highlighting study area; C: Study area. Gorakhpur division = Gorakhpur (GP), Kushinagar (KN), Deoria (DE) and Maharajganj (MA) districts. Basti division = Basti (BA, Sant Kabir

Nagar (SK), and Siddarthnagar (SN) districts. Azamgarh division = Azamgarh (AZ), Ballia (BL), and Mau (MA) districts. The map does not reflect the authors' assertion of territory or borders of any sovereign country.

Descriptive analyses

Descriptive and time series analyses were conducted in R (R Core Team, 2022) using packages plyr (Wickham, 2011), tidyverse (Wickham, 2017), DescTools (Signorell et al., 2019), lubridate (Grolemund & Wickham, 2011), irr (Gamer, Lemon, Gamer, Robinson, & Kendall's, 2012), and tseries (Trapletti & Hornik, 2016).

Frequency distributions and descriptive statistics were generated to describe sampling effort for JEV sero-surveillance (IgG and IgM antibody testing) by pig age group and district throughout the study period. Frequency distributions and descriptive statistics were generated to explore temporal and spatial patterns in aggregated (monthly and annually) IgM seroprevalence (all age groups combined), and IgG seroprevalence in each age group then in all age groups combined.

Apparent seroprevalence was adjusted to true seroprevalence to account for imperfect test specificity and sensitivity (Rogan & Gladen, 1978) with 95% confidence intervals according to Blaker's method (Blaker, 2000) in the R package, epi-R (Stevenson et al., 2017). Linear regression was used to quantify the secular trend and seasonality, followed by time series decomposition to visualize temporal components including trend, seasonality (annual cyclical variation), and the remainder, or random, component that could not be accounted for by trend or cyclical variation. A heat plot (function 'geom_tile' in R package ggplot2; Wickham (2009)) was generated to visualise the rolling mean of quarterly IgG seroprevalence (the mean IgG seroprevalence across current, prior and subsequent quarters) by district throughout the study period.

Autoregressive time-series models

A seasonal auto-regressive integrated moving average time-series (SARIMA) model was fitted to the times series of IgG seroprevalence (all age groups) for Gorakhpur district for 2013—2022. These data were selected due to geographic proximity to the Gorakhpur Station at which the climate data were recorded, and the largest proportion of samples were from this division.

Stationarity of the time series of monthly IgG seroprevalence for Gorakhpur division was assessed visually using autocorrelation function (ACF) plots and statistical tests including the Ljung-Box test for independence (null hypothesis = time independence in a given period of lags), augmented Dickey-Fuller (ADF) t-statistic test for unit root (null hypothesis = unit root present.), and the Kwiatkowski-Phillips-Schmidt-Shin (KPSS) for level and trend stationarity (null hypotheses = time-series is stationary). Following assessment of stationarity, ACF and partial autocorrelation function (PACF) plots were used to guide the manual selection of autoregressive time-series models for IgG seroprevalence, as well as automated model fitting using the 'auto.arima' function in the 'forecast' package (Hyndman & Khandakar, 2008). Fit was assessed visually using plots of residuals, and statistically by minimising Akaike's information criterion, AIC.

Cross-correlation functions were then used to assess if a statistical relationship existed between the time-series of IgG seroprevalence and monthly climate variables of total rainfall, mean relative humidity and mean minimum temperature. If correlation was detected, lagged climate variables were included in the model and fit was assessed visually using plots of residuals and statistically by minimising AIC.

Results

Surveillance effort

A total of 4451 pig serum samples were tested for IgG antibodies against JEV between 26 February 2013 and 23 December 2022. Of these, 3277 were also tested for IgM antibodies against JEV (28 October 2017 – 23 December 2022).

The annual number of samples tested for IgG (and IgM from 2017) ranged from 132—773 (Figure S1). The total number of samples tested from each district ranged from 253 in Azamgarh to 783 in Gorakhpur

(Figure S2) with the greatest number overall from Gorakhpur division (n = 2569; 58%). In each age group of 4-5 months, 6-12months and >12 months, the number of samples collected was 1049, 1967, and 1435, respectively (Figure S3).

Seroprevalence

The aggregated seroprevalence of IgG for the entire study region throughout the complete study period (2013-2022) was 14% (95% CI 12.8—15.2). The study region seroprevalence of IgM was 10.98% (95% CI 9.8—12.2) for the 3,277 pig samples tested for IgM from 2017—2022. During this same reduced period, the study region IgG seroprevalence was 9.73% (95% CI 8.5—11.0). For each age group during this period, the agreement (Cohen's *kappa* , $\varkappa$) between IgG and IgM tests was 0.60, 0.41, and 0.59 in pigs aged 4-5 months, 6-12 months, and >12 months, respectively.

Sant Kabir Nagar had the highest seroprevalence of IgG (25.4%; 95% CI 20.8—30.4), and Maharajganj had the lowest seroprevalence of IgG (4.4%; 95% CI 2.2—6.9), aggregated throughout the study period (Figure S4). Seroprevalence of IgG did not increase with age across the study region (Figure S5). Furthermore, when the data were stratified by district, no consistent pattern emerged in the point estimates of seroprevalence, with only Azamgarh, Ballia and Kushinagar demonstrating a moderate increase with age. (Figure 2). The lack of consistent increasing seroprevalence with age suggests that IgG antibodies induced following natural infection with JEV are transient, so further analyses of IgG seroprevalence were conducted stratified by pig age to investigate this phenomenon further (Sections 3.3.2-3.3.3), followed by analysis of the entire IgG dataset (all ages: Section 3.3.4).

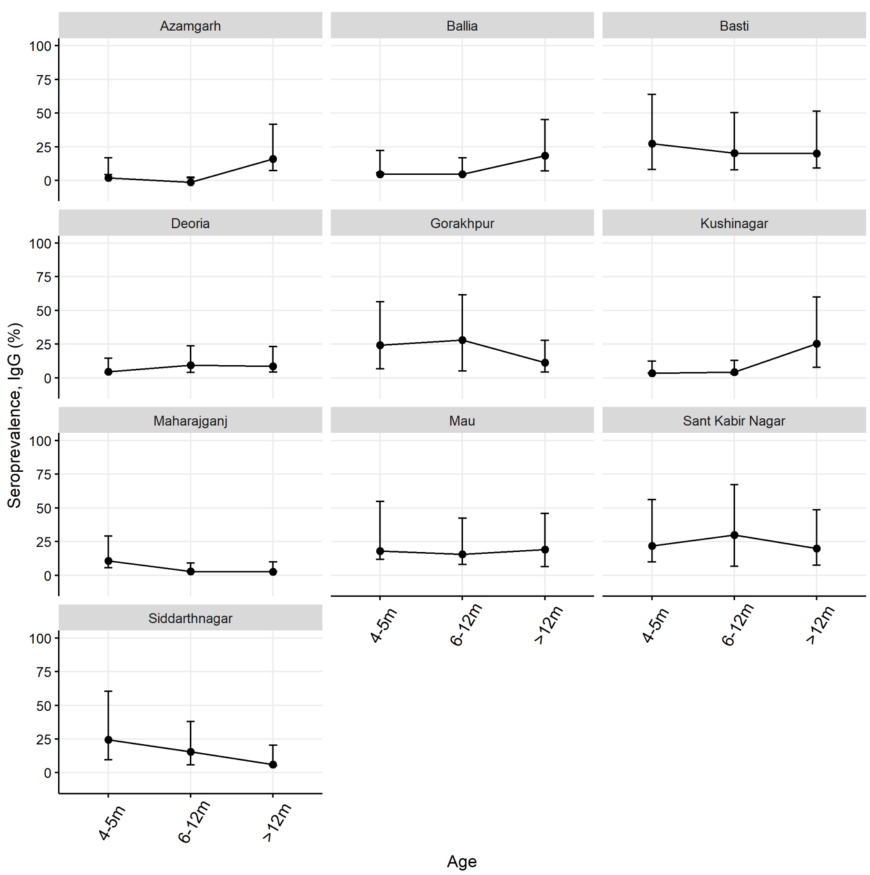

Figure 2 Seroprevalence of IgG for Japanese encephalitis virus by district and age group in blood samples

collected from pigs in eastern Uttar Pradesh, February 2013 – December 2022. Bars indicate 95% confidence intervals.

Temporospatial analyses

IgM seroprevalence all pigs 2017-2022

The number of samples tested for IgM from 28 October 2017 – 23 December 2022 was highest in Gorakhpur division (Gorakhpur, Kushinagar, Deoria and Maharajganj districts, n = 1781; 54%; Figure S6). Throughout the study period, the district with the highest IgM seroprevalence was Basti (19.4%; 95% CI 26.3-13.6), closely followed by Azamgarh and Ballia, and the lowest was Maharajganj (4.8%; 95% CI 2.7-7.8; Figure S7). Annual peak seroprevalence varied between districts, although peaks appeared consistently in 2018 in the northern and western districts (Basti, Kushinagar, Maharajganj, Sant Kabir Nagar, Siddarthnagar; Figure 3) as well as potentially the western district of Azamgarh (serology from 2018 only). The highest annual peak was observed in Gorakhpur in 2017 (69.5%; 95% CI 40.4-89.9%). Seroprevalence of IgM was similar between age groups, and a pattern of seroprevalence by age between districts was not observed (Figure S8).

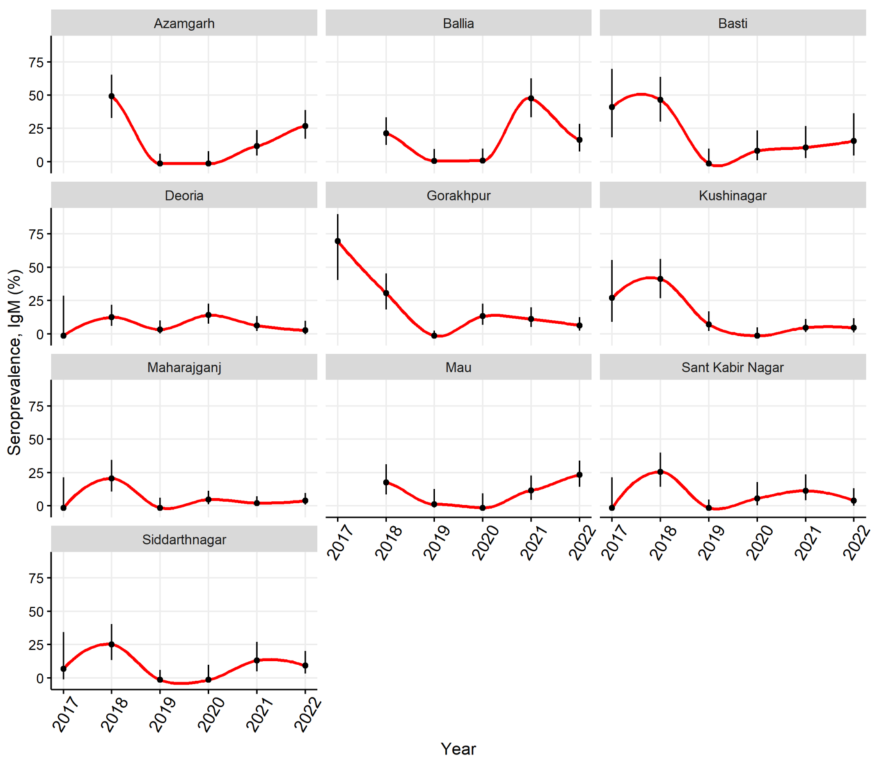

Figure 3 Annual seroprevalence of IgM for Japanese encephalitis virus in pigs in eastern Uttar Pradesh by district, October 2017 – December 2022. Bars indicate 95% confidence intervals.

Red line = loess smoothed seroprevalence.

No significant linear trend was observed in the time series of monthly IgM seroprevalence (overall change in seroprevalence = -2.1%/month [95% CI -4.5—0.4], P = 0.10). Time series decomposition revealed a marked seasonal pattern consisting of four annual peaks with the highest peak in August – September (monsoon season). However, the greatest variation in seroprevalence was due to the remainder (random) portion of the decomposed time series (range -22.3—43.7% adjustment to trend line).

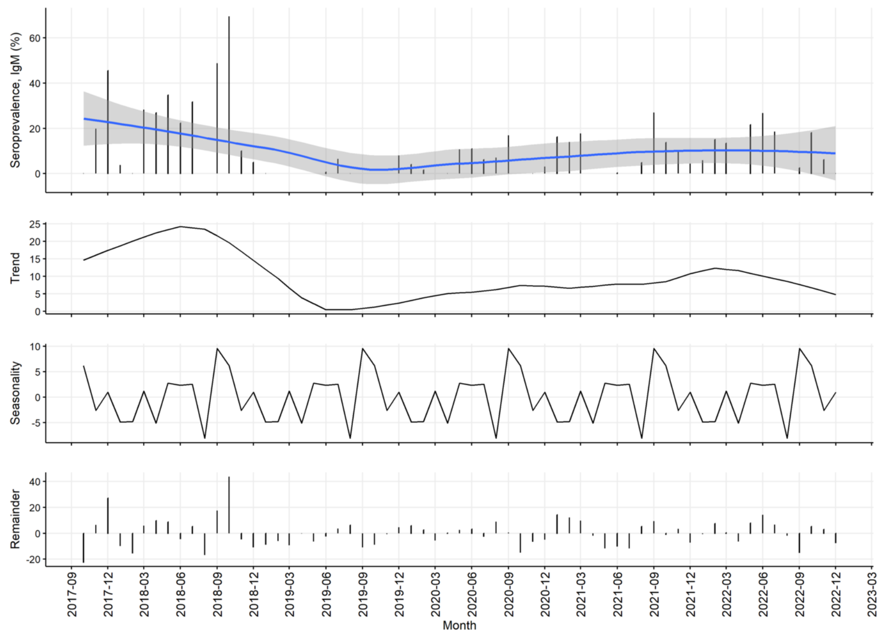

Figure 4 Time series and decomposition of monthly Japanese encephalitis virus IgM seroprevalence in pigs in eastern Uttar Pradesh, India, October 2017 – December 2022.

IgG seroprevalence by age group, 2013-2022

The number of samples tested for IgG in pigs (4-5m) was highest in Gorakhpur division (n = 644; 61%; Figure S9). The highest and lowest recorded IgG seroprevalences in young pigs throughout the study period were 27.2% (95% CI 19.1-36.7) and 2% (95% CI 0.00-14.7) in Basti and Azamgarh, respectively (Figure S10). Annual seroprevalence of IgG varied throughout the study period in all districts (Figure S11).

No significant linear trend was observed in the time series of monthly IgG seroprevalence in young pigs (overall change in seroprevalence = -1.3%/month [95% CI -3.2—0.5], P = 0.15; Figure 5). Time series decomposition again demonstrated a marked seasonal pattern with three annual peaks in March, May, and September (summer and monsoon seasons; Figure 5). Consistent with IgM seroprevalence, the greatest variation in IgG seroprevalence in young pigs was due to the remainder (random) portion of the decomposed time series.

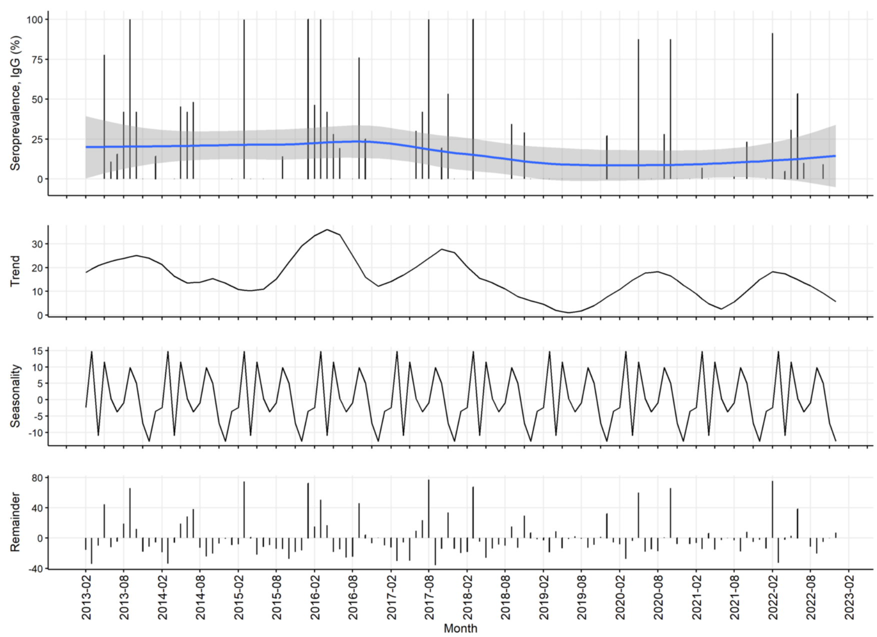

Figure 5 Time series and decomposition of monthly Japanese encephalitis virus IgG seroprevalence in pigs aged 4-5 months in eastern Uttar Pradesh, India, February 2013 – December 2022.

Time series of IgG seroprevalence in pigs aged 6—12 and [?]12 months demonstrated similar patterns to IgG seroprevalence in pigs 4-5 months (Figures S12-13). In pigs 6-12 months, the IgG seroprevalence trend was statistically significantly reduced (-2.2%/month, 95% CI -3.4—-0.5, P = 0.01), and three peaks of IgG seroprevalence were observed in January – March, May, and September (Figure S12). In pigs >12 months, the trend was not significant (-0.6%/month, 95% CI -2.5—1.2, P = 0.51), and peaks of IgG seroprevalence were observed in April, then July-December (Figure S13).

IgG seroprevalence in all pigs 2013-2022

Time series of IgG seroprevalence in all pigs is shown in Figure 6. The trend in IgG seroprevalence was statistically significantly reduced over the study period (-2.1%/month, 95% CI -3.6—-0.5, P < 0.01), and annually, with peaks of IgG seroprevalence apparent from January – March (two small peaks) and May – September (two larger peaks). Consistent with IgM seroprevalence, most variation was due to the remainder (random) component in the time series (range -32.6—76.9).

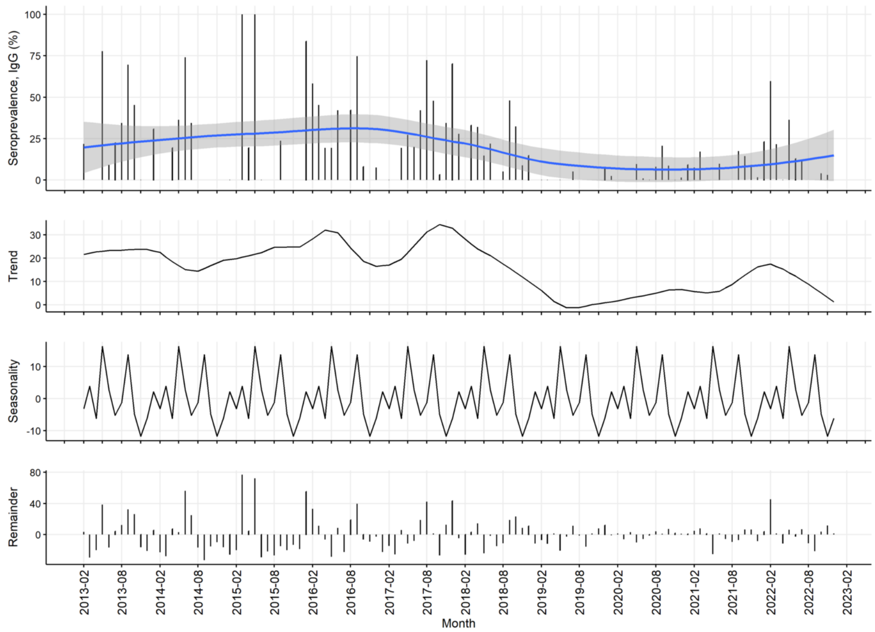

Figure 6 Time series and decomposition of monthly Japanese encephalitis virus IgG seroprevalence in all pigs sampled in eastern Uttar Pradesh, India, February 2013 – December 2022.

Annual peaks of IgG seroprevalence were highest in Sant Kabir Nagar and Gorakhpur (60.8% in 2016 and 57.0% in 2015, respectively; Figure S14). A pattern of annual IgG seroprevalence by district was not apparent (Figure S14) and was further investigated using the rolling mean of the quarterly seroprevalence (IgG) of JEV in pigs across 10 districts of eastern Uttar Pradesh under the study period (Figure 7). Periods of low then slowly increasing seroprevalence occurred across all districts except Deoria in the second half of the study period from 2019—2022. In the first half of the study period from 2014—2018, the rolling quarterly IgG seroprevalence was overall higher, and this appeared to be predominantly in districts prone to flooding (Kushinagar, Siddarthnagar, Gorakhpur, Basti and Sant Kabir Nagar).

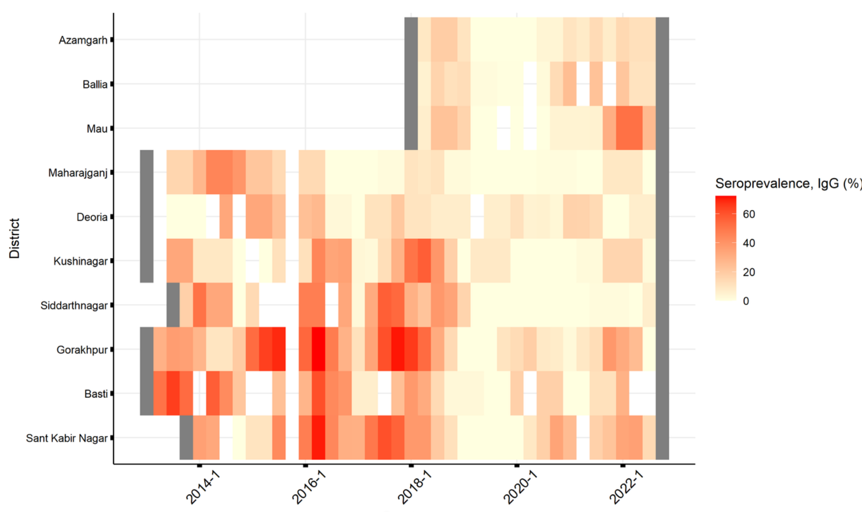

Figure 7 Heatplot of rolling quarterly mean Japanese encephalitis virus IgG seroprevalence in all pigs

sampled in eastern Uttar Pradesh, India, February 2013 – December 2022.

Autoregressive time-series models

The autocorrelation function (ACF) plot (Figure S15) and statistical tests for the monthly seroprevalence of pigs of all age groups sampled in Gorakhpur district (n = 783) were consistent with stationarity (Box-Ljung $X^2 = 13.2$, P = 0.35; ADF = -3.6, P = 0.03; KPSS Trend = 0.14, P = 0.05; KPSS Level = 0.28, P = 0.1). However, using systematic combinations of seasonality and differencing, the best fitting model, with relatively low AIC (1129), symmetrical residuals, with no autocorrelation demonstrated in the ACF plot was a model of order (p = 5, q = 1, P = 2, Q = 1) which incorporated seasonality (12) and differencing (d = 1, D = 1; Table 1; Figure S16). Trend lines of monthly total rainfall, mean minimum temperature, and mean relative humidity showed that highest rainfall and humidity was in the second half of the study period, and peaks in mean monthly temperature occurred in 2016 and 2022 (Figure S17). Cross-correlation function plots indicated no relationship between JEV seroprevalence and either monthly total rainfall or mean minimum temperature (Figures S18 and S19) but did indicate that JEV seroprevalence was negatively correlated with mean relative humidity at 6 months lag (Figure S20). Humidity was included with increasing lags in the ARIMA model (Model 4, Table 1). The model with the lowest AIC (1004.91) incorporated mean relative humidity at 12 months lag (residuals Figure S21) suggesting an inverse relationship between humidity and IgG seroprevalence in pigs. This likely reflects the broad pattern of higher IgG seroprevalence in Gorakhpur in the first half of the study period when humidity was lowest, and the reverse of this (seroprevalence low, humidity high) in the second half of the study period.

Discussion

This study provides the first long-term analysis of JEV seroprevalence in a domestic pig population, and is in a region in India – eastern Uttar Pradesh – in which a high incidence of cases in people occurs annually (A. K. Singh et al., 2020). A key finding was that IgG seroprevalence did not increase with age, indicating that IgG could be transient, and also that JEV infection might not be an immunising infection in pigs.

IgM is an indicator of recent JEV infection in pigs; IgM can be detected 4—7 days post-infection, peaking at approximately 2 weeks and declining over the following 4 weeks (H. Dhanze, Kumar, et al., 2020). If JEV infection was immunising, a reduction in IgM seroprevalence would have been consistently observed with age, even if pigs were not relying on a humoral response to JEV involving IgG. In a review of JEV epidemiology in pigs, Ladreyt et al. (2019) reported that in experimental studies, antibodies to JEV were detected for at least 28 days post-inoculation in two experimental studies, and a study of piglets under field conditions found immunity for up to 3 years (Geevarghese, Shaikh, Jacob, & Bhat, 1994). In this latter study, it was possible that the pigs had been repeatedly infected with JEV and that cross-reaction between antibodies to other flaviviruses (for example, West Nile virus) had occurred. In an experimental case study, IgG was found to decline after 40 days and remained at low levels at one year in a single 100-day old pig experimentally injected with purified recombinant NS1 protein as a JEV associated antigen, but this might not be representative of the immune response following natural JEV infection (H. Dhanze, Kumar, et al., 2020). In a field study of JE in Sri Lanka (Peiris et al., 1993), point estimates of age-stratified prevalence of neutralising antibodies decreased between 7-12 month and 13-24 month pigs at one site (48% [90% CI 29-67] and 19% [90%CI 12-28], respectively), and were similar at others (for example, 21% [90% CI 7-47] and 33% [90%CI 11-65], respectively). It is possible that ages of pigs in the current study were misclassified, but we believe this would be a small number because samples were taken by veterinarians familiar with pig breeds in the region. It is also possible that further stratification within districts would demonstrate increasing IgG seroprevalence with age if JEV transmission has consistent localised patterns within districts. Therefore, we recommend that longitudinal studies of IgG levels in individuals in pig populations at continuous risk of JEV infection should be undertaken to determine whether our hypothesis is correct. There are public health implications where intervention strategies have been based on models in which persistent immunity in pigs was assumed; the force of infection on people from pigs might be higher than estimated if susceptible pigs are not only replenished through births and young pigs losing maternal immunity, but also adult pigs losing acquired immunity.

The district-level spatio-temporal pattern of JEV IgG or IgM seroprevalence did not follow a discernible pattern, which is consistent with endemicity – waves did not reliably occur simultaneously in districts across the region, and neither was a wave of spread apparent from one district to the next. Seroprevalence of IgM was found year-round, indicating a subtropical epidemiological pattern of JEV transmission (Suresh et al., 2022). The seroprevalence throughout the region was consistent with previous, shorter duration studies, with large peaks observed, such as the annual seroprevalence of 60.8% in Sant Kabir Nagar in 2016 and 69.5% in Gorakhpur in 2017 for IgG and IgM, respectively, and some region-wide monthly IgG seroprevalence peaks reaching 100% (H. Dhanze et al., 2014; Kolhe et al., 2015). Decomposition of the monthly time series of seroprevalence for IgG and IgM across all age groups demonstrated that most variance in JEV seroprevalence was due to the unexplained 'random' portion of the time-series, followed by the underlying trend. Although annual cyclical waves with 3-4 peaks annually were apparent in the decomposition, the variance of these waves was relatively small. These cyclical waves are also consistent with the expected dynamics of an endemic infectious disease, in which regular epidemic waves occur as the proportion of susceptible pigs in a population increases through births – it does not indicate necessarily that an infection is non-immunising although this can contribute to the effect. Generally, seroprevalence peaks were higher, or arose from a higher baseline, in the monsoon period, indicating a seasonality which has been shown previously (Borah et al., 2013; Kumari & Joshi, 2012; Pantawane et al., 2017; A. K. Singh et al., 2020). In the monsoon season, temperature is warmer and rainfall is higher, providing mosquitoes with more breeding habitat and promoting shorter developmental cycles. The predominant vector of JEV, *Culex tritaeniorhynchus* , has been shown to be most abundant in the monsoon season in a previous study in Gorakhpur district (Kanojia, Shetty, & Geevarghese, 2003).

Because most variance in the time series decomposition was due to the random (unexplained) portion, autoregressive modelling of JEV IgG seroprevalence in Gorakhpur district was not sufficiently nuanced to be usefully predictive. Interestingly, the underlying trend of IgG seroprevalence in Gorakhpur – higher overall seroprevalence in the first half of the study period, similar to the region-wide trend – was the inverse of the monthly mean relative humidity and total rainfall which were both higher in the second half of the study period. This was reflected in the best fitting model in which mean monthly relative humidity was negatively correlated with seroprevalence. Previous studies have stated that pigs could be used as sentinels for outbreaks of JEV in people (Suresh et al., 2022). Given that seroconversion in pigs can be highly correlated with cases in people 2-4 weeks later (study in Assam; Borah et al. (2013), increased seroprevalence in pig populations could theoretically be used as a predictor of human cases. Whilst the utility of this might be useful for more immediate control options such as fogging in areas detected with high seroprevalence in pigs, the utility of this is low for predictive surveillance due to timeliness. Collection of blood samples, analysis and reporting, as well as implementation of longer-term control vaccination programs in people and the time required to develop immunity would be greater than 2-4 weeks.

A strength of this study included the relatively large dataset over a long time period, enabling comprehensive descriptive analysis of the seroprevalence of pigs in this region. However, limitations included sparse data in some districts, and the lack of longitudinal analysis of pigs. In addition, reported cases in people from the same districts during the study period would enable further evaluation of the value of sero-surveillance in pigs as part of a public health surveillance program.

Cases in people in Uttar Pradesh generally occur from June and peak in September—October (Kumari & Joshi, 2012). This study demonstrates that for sero-surveillance in pigs to be useful to predict the onset and magnitude of human cases at district level so that public health resources for interventions including vaccination programs, mosquito control and bite prevention can be targeted to the highest risk districts, more detailed data about known risk factors is needed at local scales. It is possible that other risk factors such as local availability of mosquito breeding habitat, ground surface temperature, water bird abundance and other landscape factors might be useful in predicting both pig and human cases. Pig sero-surveillance could have a role in confirming predictions and the need for maintenance of interventions in high risk periods for people.

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Conflict of Interest Statement

The authors declare no conflicts of interest.

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Table 1 Model specifications for seasonal autoregressive time series models of JEV seroprevalence in pigs sampled in eastern Uttar Pradesh, India, February 2013 – December 2022. AIC = Akaike information criterion. Final model = model 6.

Model	Non-seasonal terms	Non-seasonal terms	Non-seasonal terms	Seasonal terms	Seasonal terms	Seasonal term
	p	d	q	P	D	Q
1	1	0	2	-	-	-
2	0	0	2	2	**1**	0
3	0	**1**	1	-	**-**	-
4	5	**1**	1	2	**1**	0
5	**5**	**1**	**1**	**2**	**1**	**1**