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Preplanned Studies

Assessment on the Diagnostic Capacity for Parasitic Diseases of Health Facilities — China, 2019

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Summary

What is already known about this topic?
Morbidity and prevalence of several major parasitic diseases have been declining in China. To reduce the disease burden of parasitic diseases and protect public health security, conducting accurate diagnoses following timely treatment is important.

What is added by this report?
In the national competition held in 2019, the overall accuracy rates of participants for theoretical knowledge and slides interpretation for parasitic diseases were 80.44% and 66.87%, respectively. Significant differences in the accuracy rates of detecting schistosomiasis or malaria existed between endemic areas and non-endemic areas, respectively.

What are the implications for public health practice?
The study results will help policymakers and health managers to identify the gaps in parasitic diseases, help to strengthen diagnostic capacity, and improve quality of control programs.

China used to be overburdened by the heavy burden caused by parasitic diseases. However, through several decades of effort, the number of cases and the prevalence of parasitic diseases had declined dramatically nationwide (1–3). However, misdiagnosis of parasitic diseases was reported from occasionally, especially for imported cases or asymptomatic cases with light infections. To understand the gap in diagnostic capacity that exists for parasitic diseases, a nationwide competition was conducted in August 2019 by the National Health Commission (NHC) through a paper test and interpretation of slides containing parasite worms or eggs. Generally, technicians grasped the knowledge of parasitic disease well but their practical skills for slides interpretation were not satisfactory with an overall accuracy rate of 66.87%. As parasitological methods relying on interpreting slides were still the gold standard for diagnosing parasitic diseases, further capacity building for diagnosis, especially parasitological methods, should be strengthened to avoid misdiagnosis of patients and provide accurate data for policymaking.

In August 2019, a national competition for parasitic diseases was held by the NHC in Changchun City of Jilin Province. A total of 32 teams with 128 technicians attended the competition. Each team was composed of four professional staff members, with at least one being from hospitals and two from agencies below the provincial level. All participants were aged less than or equal to 45 years old. The competition consisted of 2 parts: 1) theoretical knowledge on the life cycle of common parasites, their etiology and diagnosis, and control and prevention strategies of several parasitic diseases were evaluated through a paper test composed of 50 questions featured as single-choice, multiple-choice, and true or false categories; and 2) the technical skills for interpretation of blood smear slides and Kato-Katz thick smear slides were examined. All slides and the reference answers were prepared by the National Institute of Parasitic Disease (NIPD) of China CDC.

All assessment activities were conducted in parallel in single blind manner. The original results of each participant were entered into a computer by Microsoft Excel (version 2013, Microsoft Corp, Redmond, USA) and descriptive statistics was conducted by SPSS software (version 20, IBM Corp, Armonk, USA) to describe the general information of participants. The accuracy rate was calculated as the number of questions answered accurately divided by the total number of questions, or the accumulated number of parasite species in slides interpreted accurately divided by the total number of referenced parasite species provided by NIPD. Chi-squared statistics were used to compare the values between or among subgroups. The level of statistical significance was defined as \( P<0.05 \).

In total, 128 participants from 30 provincial-level administrative divisions (PLADs), Xinjiang Production and Construction Corps, and Chinese General Administration of Customs attended the competition.
Overall, 28.9% (37/128) of participants were male and 71.1% (91/128) were female. All participants were aged with a mean value of 32.4±4.95 years old. The majority of participants (96.1%, 123/128) were junior staff with primary or middle level professional titles, while the rest were senior staff. Among of them, 82 participants were working at agencies of public health while 46 participants were from hospitals.

In the theoretical knowledge assessment, the accuracy rate was 80.44% (5,148/6,400) for all participants but varied from 38.00% (19/50) to 98.00% (49/50). Chi-square analysis showed that the females had higher accuracy rates than the males ($\chi^2=14.139, P<0.01$) while the accuracy rate in participants from public health agencies was higher than that from hospitals ($\chi^2=8.374, P<0.01$). Significant differences were also detected when analyzing accuracy rates by strata of professional titles and agencies ($\chi^2=8.571, \chi^2=24.023, P<0.05$). But no difference in accuracy rate was found among different age groups ($\chi^2=2.442, P>0.05$) (Table 1).

For the knowledge points, the accuracy rates for detecting $P$. falciparum, $P$. vivax, and negative slides were 57.50% (23/40), 84.38% (1,080/1,280), 83.98% (1,075/1,280), 76.72% (982/1,280), 81.75% (1,465/1,792), and 71.09% (546/768), respectively, showing significant difference ($\chi^2=78.673, P<0.05$).

For the interpretation of slides, the accuracy rate for thick smear slides and blood smear slides was 67.19% (2,097/3,121) and 65.31% (418/640), respectively, with the overall accuracy rate being 66.87% (2,515/3,761) (Table 2). Individually, the accuracy rate for the interpretation of thick smear slides ranged from 10% (3/30) to 96.55% (28/29); 22 participants had a 100% accuracy rate, but 4 participants provided all wrong answers for blood smear slides interpretation.

The average accuracy rates did not differ significantly among agencies ($\chi^2=5.494, \chi^2=0.073, P>0.05$), but females, younger professional staff, and senior staff had higher accuracy rates ($P<0.05$).

In analyzing the accuracy rates by helminth species, the highest accuracy was 87.50% (420/480) for the detection of Clonorchis sinensis, followed by 83.73% (669/799) for Ascaris spp. The lowest accuracy rate was 25.89% (124/479) for detection of Schistosoma japonicum (Table 3). Significant differences in accuracy rates were detected among helminth species identification ($\chi^2=593.544, P<0.05$). For identification of plasmodium species, the accuracy rate for detecting Plasmodium malariae, P. falciparum, P. vivax, and negative slides were 57.50% (23/40), 60.47% (179/296), 69.64% (117/168), and 72.79% (99/136), respectively, presenting significant differences ($\chi^2=8.888, P<0.05$). The accuracy rate of

| Categories | Features | No. participants | No. questions answered | No. questions answered accurately | Accuracy rate (%) | Chi-square test |
|------------|----------|------------------|------------------------|------------------------------------|-------------------|----------------|
| Total      |          | 128              | 6,400                  | 5,148                              | 80.44             |                |
| Gender     | Male     | 37               | 1,850                  | 1,434                              | 77.51             | $\chi^2=14.139, P<0.001$ |
|            | Female   | 91               | 4,550                  | 3,714                              | 81.63             |                |
| Age, years | ≤30      | 43               | 2,150                  | 1,706                              | 79.35             |                |
|            | 31–40    | 72               | 3,600                  | 2,915                              | 80.97             | $\chi^2=2.442, P=0.295$ |
|            | ≥41      | 13               | 650                    | 527                                | 81.08             |                |
| Professional title | Primary      | 74               | 3,700                  | 2,933                              | 79.27             |                |
|            | Middle   | 49               | 2,450                  | 2,004                              | 81.80             | $\chi^2=8.571, P=0.014$ |
|            | High     | 5                | 250                    | 211                                | 84.40             |                |
| Agency level | Province    | 19               | 950                    | 709                                | 74.63             |                |
|            | City     | 40               | 2,000                  | 1,624                              | 81.20             | $\chi^2=24.023, P<0.001$ |
|            | County   | 69               | 3,450                  | 2,815                              | 81.59             |                |
| Agency feature | Public health | 82              | 4,100                  | 3,342                              | 81.51             | $\chi^2=8.374, P=0.004$ |
|            | Hospital | 46               | 2,300                  | 1,806                              | 78.52             |                |
participants from schistosomiasis endemic areas for schistosomiasis slides interpretation was 35.87% (66/184), significantly higher than that of 19.66% (58/295) in participants from non-endemic areas ($\chi^2=15.517, P<0.01$). Similarly, the accuracy rate of participants from malaria endemic areas for malaria slides interpretation was higher than that of participants from non-endemic areas [70.00% (322/460) vs 53.33% (96/180), $\chi^2=15.863, P<0.01$].

**DISCUSSION**

The results from the assessment through nationwide competition on the diagnosis of parasitic diseases showed that technicians grasped the knowledge of parasitic disease generally well, but the interpretation of slides of parasitological methods were not satisfactory. Females demonstrated higher diagnostic capacity than males, but differences of accuracy rates in

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**TABLE 2.** The accuracy rates of participants for slides interpretation in China, 2019.

| Categories | Features | No. participants | Accumulated slides number | Accumulated slides number judged parasites correctly | Accuracy rate (%) | Chi-square test |
|------------|----------|------------------|----------------------------|-----------------------------------------------------|------------------|----------------|
| Total      |          | 128              | 3,761                      | 2,515                                               | 66.87            |                |
| Gender     | Male     | 37               | 1,082                      | 688                                                 | 63.59            | $\chi^2=7.397, P=0.007$ |
|            | Female   | 91               | 2,679                      | 1,827                                               | 68.20            |                |
| Age, years | ≤30      | 43               | 1,268                      | 896                                                 | 70.66            |                |
|            | 31–40    | 72               | 2,114                      | 1,377                                               | 65.14            | $\chi^2=12.655, P=0.002$ |
|            | ≥41      | 13               | 379                        | 242                                                 | 63.85            |                |
| Professional title | Primary | 74               | 2,173                      | 1,477                                               | 67.97            |                |
|            | Middle   | 49               | 1,441                      | 930                                                 | 64.54            | $\chi^2=7.614, P=0.020$ |
|            | High     | 5                | 147                        | 108                                                 | 73.47            |                |
| Agency level | Province | 19               | 559                        | 351                                                 | 62.79            |                |
|            | City     | 40               | 1,171                      | 801                                                 | 68.40            | $\chi^2=5.494, P=0.064$ |
|            | County   | 69               | 2,031                      | 1,363                                               | 67.11            |                |
| Agency feature | CDC    | 82               | 2,423                      | 1,624                                               | 67.02            | $\chi^2=0.073, P=0.787$ |
|            | Hospital | 46               | 1,338                      | 891                                                 | 66.59            |                |

Note: CDC: Center for Disease Control and Prevention.

**TABLE 3.** The accuracy rates of 128 participants for parasites identification based on slides interpretation in China, 2019.

| Types of slides | Types of parasites in slides | Accumulated number of slides | Accumulated number of slides judged correctly | Accuracy rate (%) |
|-----------------|------------------------------|------------------------------|---------------------------------------------|------------------|
| Total           |                              | 3,761                        | 2,515                                       | 66.87            |
| Thick smear slides | Schistosoma japonicum | 479                          | 124                                        | 25.89            |
|                  | Trichuris trichiura         | 161                          | 97                                         | 60.25            |
|                  | Paragonimus                 | 320                          | 206                                        | 64.38            |
|                  | Fasciolopsis                | 80                           | 64                                         | 80.00            |
|                  | Ascarias spp.               | 799                          | 669                                        | 83.73            |
|                  | Enterobius vermicularis     | 241                          | 183                                        | 75.93            |
|                  | Clonorchis sinensis         | 480                          | 420                                        | 87.50            |
|                  | Taeniidae                   | 240                          | 142                                        | 59.17            |
|                  | Spirometra mansonia         | 321                          | 192                                        | 59.81            |
|                  | Subtotal                    | 3,121                        | 2,097                                      | 67.19            |
| Blood smear slides | Plasmodium falciparum | 296                          | 179                                        | 60.47            |
|                  | Plasmodium vivax            | 168                          | 117                                        | 69.64            |
|                  | Plasmodium malariae         | 40                           | 23                                         | 57.50            |
|                  | Negatives                   | 136                          | 99                                         | 72.79            |
|                  | Subtotal                    | 640                          | 418                                        | 65.31            |
participants also existed among subgroups when analyzing the data by professional title, specific knowledge points, or species of parasites. Significant difference in accuracy rates of detecting schistosomiasis and malaria was also detected between endemic areas and non-endemic areas, respectively.

With the decline of prevalence and infection intensity of local parasitic diseases and increase in the number of imported or emerging parasitic diseases (4–6), misdiagnosis occurred occasionally due to declining conscientiousness or weak skills related to parasitic diseases. The evaluation conducted nationwide by the NHC during 2012–2016 indicated gaps in the diagnosis of parasitic diseases nationwide to control or eliminate parasitic diseases (7). In past decade, capacity building was strengthened through construction of reference laboratories for parasitic diseases, conducting of training courses, or organizing inter-laboratories comparison (8–9). In this study, we found the diagnostic capacity for parasitic diseases improved but gaps still existed.

From the knowledge test results, the average accuracy rate was higher than 80%, demonstrating a general understanding of knowledge toward parasitic diseases. However, the accuracy rates differed significantly among individuals in the range of 38%–98%. Males, staff with first-level professional titles, and staff from hospitals presented lower accuracy rates for knowledge tests. In addition, the accuracy rates of questions among knowledge points and categories of diseases were relatively low in questions related to echinococcosis, comprehensive questions, and the control and prevention of parasitic diseases. The average accuracy rate for the interpretation of slides was 66.87% (2,515/3,761), suggesting big challenges existed in the diagnosis of parasitic diseases. For determination of parasitic species, the accuracy rates were highest for Clonorchis sinensis with an accuracy rate of 87.50%, which may be explained by increased attention on liver diseases in recent decades and the typical features of eggs that can be easily identified (10). The accuracy rates for 2 major parasitic diseases in China including malaria and schistosomiasis were 65.31% and 25.89%, respectively. Significant differences in accuracy rates existed between endemic and non-endemic area of schistosomiasis and malaria. As malaria has been eliminated in 2021 and schistosomiasis is close to elimination in China, the gap existing in diagnosis should be filled to build capacity building for the prompt finding of endemic cases and imported cases from foreign countries.

There are several limitations in this study. One limitation is that the participants involved in this study only accounted for a small part of medical workers in China. To understand the overall capacity level for diagnosis of parasitic diseases, further assessment should be conducted extensively. The other one is that the questionnaire test and slides used hadn’t received scientific assessment. In addition, the results for both parts were analyzed separately and couldn’t assess the overall capacity of participants comprehensively. It should be noted that all participants in this competition were recommended or selected by each PLAD and possibly received long periods of training. The actual diagnostic capacity in health facilities across China may be much lower than that reflected in our study and likely could not meet the needs of national control and prevention strategies for parasitic diseases. Further capacity building should be enhanced at all levels through more training courses, especially for parasitological tests as they remain the diagnostic gold standard of parasitic diseases. To ensure the efficiency of diagnosis, external quality assessment and rechecking samples should be considered and conducted during the implementation in the national control programs.

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Modelling the Ecological Suitability of the Chagas Disease Vector
*Triatoma rubrofasciata* — China

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Summary

What is already known on this topic?

*Triatoma rubrofasciata* is a potential vector that can transmit American trypanosomiasis and was widely recorded in South of China.

What is added by this report?

Because of the low density of the triatomines, more habitats have not been discovered. This study mainly focused on predicting the geographical distribution of *T. rubrofasciata* under current and future climatic conditions in China using the MaxEnt model.

What are the implications for public health practice?

The result showed that the distribution of *T. rubrofasciata* was largely affected by annual mean temperature and minimum temperature of the coldest month. According to species response curves, this species preferred habitats with annual mean temperatures of 22.5 °C. Climate change projections indicated that by 2050 and 2070, the habitats of the *T. rubrofasciata* would be expanded.

*T. rubrofasciata* is one of the potential vectors of Chagas disease that is recorded to be the most widely distributed worldwide. In China, we started monitoring triatomines and imported Chagas disease in 2016. During a survey from 2016–2018, 170 habitats were recorded (3), but because of low density of the triatomines, many habitats were likely underrepresented. Therefore, predicting these habitats through appropriate environmental data is important to make up for this underrepresentation.

MaxEnt is a machine learning model that estimates a target probability distribution by calculating the probability distribution of maximum entropy (4). MaxEnt has been used extensively to model the distribution of several vector-reliant disease-causing pathogens (5–6). Many scholars believed that the MaxEnt model was better than other models in predicting the spatial distribution of species, especially in cases of incomplete distribution data (6–7). MaxEnt is also capable of projecting shifts in species distribution under various climate change scenarios (4).

In this study, MaxEnt software was used to predict the potential distribution of *T. rubrofasciata*. A total of 184 records have been collected in the database. These came from two sources: 1) published records on *T. rubrofasciata* in journals, books, and reports and from Shanghai Insect Museum and Museum of insects Institute of Guangdong Province in China (8–11); and 2) specimen field surveys by the authors from 2016 (3). After screening duplicates and poor data, 116 records were obtained for the construction of the final model.

For present environmental data (1950–2000), a total of 19 bioclimatic layers were downloaded from Worldclim database (www.worldclim.org) (Supplementary Table S1, available in http://weekly.chinacdc.cn/). All data used for SDM had a spatial
resolution of 1 km² (30 arc seconds). The data of distribution points and environmental variables were inputted into MaxEnt software. The maximum number of iterations was set to 2000, bootstrap repeated 10 times, and random seed was turned on. Then, the response curves and jackknife test functions were checked to analyze the importance of environmental variables that affected the distribution of *T. rubrofasciata*. The establishment of the model needs to randomly select 75% of the distribution points, and the test of the model is completed by data of the remaining 25% of the occurrence points to construct an optimal habitat model of *T. rubrofasciata*. The prediction results of MaxEnt model were tested by the area under the receiver operating characteristic curve (ROC). Maximum entropy modeling was used with the MaxEnt software (version 3.3.3k, American Museum of Natural History, NY, USA) for quantifying relative risk of invasion and mapping of the potential geographic distribution of *T. rubrofasciata* in China. A jackknife procedure was used to calculate the significance of the contribution of each variable to the model. The area under the receiver operating characteristic curve [area under curve (AUC)] was used to evaluate model performance. The potential range of changes in the suitable areas under different scenarios of climate change in the future was analyzed by using the “distribution changes between binary SDMs” tool. When performing the quick recursive to binary conversion, the threshold value was 0.5. ArcMap was loaded for all maps..

The MaxEnt model prediction has high AUC (0.989) indicating good model performance for *T. rubrofasciata* (Supplementary Figure S1, available in http://weekly.chinacdc.cn/). The jackknife test showed that the distribution of *T. rubrofasciata* was largely affected by annual mean temperature (Bio1), mean temperature of the coldest quarter (Bio11), and minimum temperature of coldest month (Bio6) (Figure 1). Annual mean temperature (Bio1) explained 63.4% of the total variance and was thus identified as the main factor affecting the spatial distribution of *T. rubrofasciata*. Species distribution maps showed that Guangdong, Guangxi, Fujian, and Taiwan, China of the study area were recognized as high potential habitats of *T. rubrofasciata* in the present conditions, by 2050, and by 2070 (Figure 2). The averaged future predictions of MaxEnt for 2050 and 2070 (binary SDMs) revealed an expansion in suitable habitats of *T. rubrofasciata* of 180,433 km² and 167,495 km² respectively, which reached more northern latitudes of southern China (Figure 3).

![Figure 1](http://weekly.chinacdc.cn/)

**FIGURE 1.** Relative predictive power of different environmental variables based on the jackknife of regularized training gain in maximum entry modeling for *T. rubrofasciata* in China.
**DISCUSSION**

*T. rubrofasciata*, which was recorded as a potential vector that can transmit American trypanosomiasis, was the most widely distributed worldwide. During the survey of 2016–2018, the habitats of *T. rubrofasciata* was widely recorded in southern China, as well as exhibiting domestic and peri-domestic behavior invading chicken coops and human dwellings. However, due to its low density, more habitats would likely be underrepresented, including, for example, historical reports in Yunnan Province that were not corroborated in current surveys (2). For this purpose, we used MaxEnt to identify regions that offer climatically suitable conditions for this species.

The discriminatory capacity of the model displayed good predictive performance, which was also reflected in the AUC values of over 0.95. Studies have indicated that potential hotspots of triatomine species diversity in tropical and subtropical regions between 21°Fand 24°F latitude and a major limiting factor of triatomine distribution was temperature seasonality (12–13). In our study, after removing auto-correlated parameters (Figure 1), MaxEnt indicated that the current distribution of *T. rubrofasciata* was more affected by temperature variables (Bio1, Bio6, and Bio11). Among them, Bio1 (annual mean temperature) was the most affected and explained 63.4% of the total variance. The International Panel of Climate Change (IPCC) has predicted that annual mean temperatures will increase up to 5.8 °C by the end of this century (14). As the global climate warms, the risk of *T. rubrofasciata* expansion may increase.

The information obtained from the predictive maps of the current distribution of *T. rubrofasciata* was compared to those assembled from the series of future predictions in 2050 and 2070 with a medium GHG
concentration scenario (RCP26). Global climate models trained on the existing potential distribution showed a relatively stable estimate for the amount of land area that was classified as suitable for *T. rubrofasciata*. The two emissions scenarios we modeled (RCP 26) produced very similar predicted distributions, although the pathway of high concentrations of GHG predicted slightly less overall area (Figure 2). There was a predicted shift in the distribution with suitable areas moving from lower latitude and presumably warmer climates to climates at higher latitude where conditions may become more suitable (Figure 2). Regions of high predicted probability in 2050 were found in Guangxi, Guangdong and Hainan, and good suitable areas were expanded in Southern of Fujian, Jiangxi, Hunan, Guizhou, and Yunnan.

In general, using this model, it was easy to find climatically suitable habitats of *T. rubrofasciata* in China. It was useful for targeting surveillance and increasing the efficiency and accuracy of public health investigations and vector control efforts in China in the future. Background surveys should focus on southern China and set up more sampling survey points, while a small number of sampling survey points could be set up in the southern areas of Yunnan, Guizhou, Jiangxi, and Fujian.

However, the present study has some limitations. Although more than 100 records were obtained for the construction the model, the data was mainly concentrated in Guangdong and Guangxi, so its representation has certain limitations. More data would be collected to add to the model to get more accurate prediction. The widely used “MaxEnt” software for modeling species distributions from presence-only data tends to produce models with high-predictive performance but low-ecological interpretability, which are more complex but not necessarily predictively better-than subset selection. The simpler models implement two different kinds of model fitting: maximum entropy fitting for presence-only data and logistic regression for presence-absence data would be new motivation field. With improvement of the model, some model which could cover the shortage of the data, eg, MIAmaxent R package etc, which would be involved in the habitats of *T. rubrofasciata* prediction in future study (15).

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SUPPLEMENTARY TABLE S1. Climatic variables used for predicting the potential distribution of Chagas disease vector *Triatoma rubrofasciata* in China based on MaxEnt.

| Code | Variable | Source |
|------|----------|--------|
| Bio1 | Annual mean temperature | http://www.worldclim.org/ |
| Bio2 | Mean diurnal range | http://www.worldclim.org/ |
| Bio3 | Isothermality | http://www.worldclim.org/ |
| Bio4 | Temperature seasonality | http://www.worldclim.org/ |
| Bio5 | Max temperature of the warmest month | http://www.worldclim.org/ |
| Bio6 | Min temperature of the coldest month | http://www.worldclim.org/ |
| Bio7 | Temperature annual range | http://www.worldclim.org/ |
| Bio8 | Mean temperature of the wettest quarter | http://www.worldclim.org/ |
| Bio9 | Mean temperature of the driest quarter | http://www.worldclim.org/ |
| Bio10 | Mean temperature of the warmest quarter | http://www.worldclim.org/ |
| Bio11 | Mean temperature of the coldest quarter | http://www.worldclim.org/ |
| Bio12 | Annual precipitation | http://www.worldclim.org/ |
| Bio13 | Precipitation of the wettest month | http://www.worldclim.org/ |
| Bio14 | Precipitation of the driest month | http://www.worldclim.org/ |
| Bio15 | Precipitation seasonality | http://www.worldclim.org/ |
| Bio16 | Precipitation of the wettest quarter | http://www.worldclim.org/ |
| Bio17 | Precipitation of the driest quarter | http://www.worldclim.org/ |
| Bio18 | Precipitation of the warmest quarter | http://www.worldclim.org/ |
| Bio19 | Precipitation of the coldest quarter | http://www.worldclim.org/ |

SUPPLEMENTARY FIGURE S1. Receiver operating characteristic curve [abbreviated as area under curve (AUC)] was used to evaluate model performance by MaxEnt model.
Preplanned Studies

Surveillance of Malaria Vectors — 18 Sites, 12 PLADs, China, 2018–2020

Yuanyuan Li; Yuan Fang; Jingbo Xue; Limin Yang; Qin Liu; Wenqi Shi; Yi Zhang

Summary

What is already known on this topic?
Malaria is an infectious parasitic disease transmitted by the bite of Anopheles mosquitoes and is a serious threat to human health. Surveillance of malaria vectors is part of the integrated strategy for malaria elimination in China.

What is added by this report?
This research supplements the population distribution, density, and seasonal fluctuation of malaria vectors in the Anopheles surveillance sites from 2018 to 2020 in China.

What are the implications for public health practice?
Continuous surveillance of malaria vectors is important for maintaining malaria-free status in China and for providing a scientific basis for risk assessment of malaria retransmission.

Malaria is an infectious parasitic disease transmitted through the bite of Anopheles mosquitoes, which seriously endangers human health (1). Although significant progress has been made on malaria control in several countries, the infection is still on the list of top health threats to humans, causing 409,000 deaths worldwide in 2019 [World Health Organization (WHO), World Malaria Report 2020]. Historically, high malaria incidence rates have been reported from 24 provincial-level administrative divisions (PLADs) of China (2). Following several decades of prevention and control, the Central Government of China with strong political commitment in malaria elimination since 2010, endorsed the National Action Plan for Malaria Elimination (2010–2020) with the goal of malaria-free nationwide by 2020 (3). The last indigenous case of malaria in China was reported in April 2016 in Yunnan Province; since then, zero indigenous case has been reported in the country (4). In order to further strengthen the surveillance capacity for post-elimination phase and meet the requirement of malaria certification. Surveillance has been carried out in areas at high risk of malaria transmission based on various indicators, such as the distribution of Anopheles spp., the number of imported cases, and the risk of re-establishment of malaria transmission in China. This study aims to describe the population distribution, density, and seasonal fluctuations of Anopheles spp. at the sentinel sites (n=18) for malaria vector surveillance from 2018 to 2020. The results showed that same malaria vectors were found in the surveillance regions, but with significant discrepancy of species distribution and seasonal fluctuation. The study will help advance our knowledge in providing baseline data for the prevention and control of imported malaria cases to consolidate the achievements of malaria elimination in China.

The statistical data of this study were achieved using information from the malaria vector surveillance system in the national malaria zero indigenous case reporting phase, 2018–2020. All 18 national malaria sentinel sites in 12 PLADs of China were enrolled for malaria vectors surveillance during the malaria transmission phase (Figure 1). According to the strategy in the “National working program for malaria vector surveillance leading for malaria elimination certification in China”, lamp trapping and human-baited tent trap methods were used to monitor the population distribution, density, and seasonal fluctuation of Anopheles vectors, and quality control was carried out by Provincial and National CDCs. Microsoft Excel software (version 2016, Microsoft, USA) was used to analyze the surveillance data.

From 2018 to 2020, a total of 33,727 Anopheles spp. mosquitoes were captured by the lamp trapping method in 18 surveillance sites. From these, numbers of 31,126 An. sinensis, 1,520 An. anthropophagus, 111 An. minimus, and 970 other Anopheles spp. were identified by morphological analysis (Figure 2). An. dirus was not identified. The distribution and density of Anopheles spp. in different areas was significantly different. The proportions of An. sinensis, An. anthropophagus, and An. minimus in the total number of Anopheles spp. were 92.29%, 4.51%, and 0.33%, respectively. An.
**An. sinensis** was the most widely distributed and was detected in all areas except Motuo County, Xizang (Tibet) Autonomous Region. **An. anthropophagus** was only detected in Donggang City, Liaoning Province. **An. minimus** was found distributed in Yunnan and Guangdong Provinces.
Density and seasonal fluctuations of malaria vectors were monitored by human bait trapping methods in all surveillance sites. However, Motuo County in Xizang (Tibet) Autonomous Region was not monitored as required and was not included in the analysis. The monitoring results of the average density of malaria vectors in the surveillance sites from 2018 to 2020 were included in the density monitoring analysis and are shown in Table 1. A total of 5,481 Anopheles were captured by human bait trapping method in 17 surveillance sites, including 5,326 An. sinensis, one An. anthropophagus, 85 An. dirus, 24 An. minimus and 45 other Anopheles spp., which were different from those captured by lamp trapping method. An. anthropophagus was only detected in Congjiang County, Guizhou Province; An. dirus was detected in Wuzhishan City, Hainan Province; and An. minimus was detected in Hainan, Yunnan, and Guangdong Provinces. From 2018 to 2020, the average density of captured malaria vectors was significantly different among the surveillance sites, with the distribution range ranging from 0 to 9.15 (numbers per man per hour). Anhui and Henan provinces had the highest density distribution of Anopheles spp. Among 17 surveillance sites in 11 PLADs from 2018 to 2020, Zhejiang Province had only 1 An. sinensis, Wuzhishan City of Hainan Province had the peak malaria vectors density in May, Tengchong City and Yingjiang County of Yunnan Province, Tianya District of Hainan Province, Donggang City of Liaoning Province, and Congjiang County of Guizhou Province had peak malaria vector abundance in August, and the remaining 10 surveillance sites had a peak in Anopheles abundance between June and July.

**DISCUSSION**

The results from this study showed that An. sinensis, An. anthropophagus, An. minimus, and An. dirus were still the main malaria vectors at the stage of zero indigenous malaria case reported in China (5). The same malaria vectors existed with significant discrepancy of species distribution and seasonal fluctuation in the surveillance regions. There have been no reports of indigenous malaria infection in China for 4 consecutive years since 2017 (6). However, with the intensification of globalization, the number of people coming from or returning from malaria-endemic areas

### TABLE 1. Results of malaria vector average density surveillance by the human bait trapping methods in 17 sentinel sites, 11 PLADs, China, 2018–2020.

| PLADs | Surveillance site | Average density (number/person•hour) |
|-------|------------------|-------------------------------------|
|       | Early May | Late May | Early Jun | Late Jun | Early Jul | Late Jul | Early Aug | Late Aug | Early Sep | Late Sep | Early Oct | Late Oct |
| Anhui | Feidong     | 1.53     | 1.80     | 4.87     | 6.83     | 4.32     | 5.65     | 2.90     | 1.98     | 2.40     | 1.50     | 0.70     | 0.99     |
|       | Shucheng    | 0.00     | 0.01     | 0.33     | 0.35     | 1.64     | 1.67     | 1.25     | 0.52     | 0.14     | 0.04     | 0.06     | 0.03     |
| Yunnan | Tengchong  | 0.00     | 0.00     | 0.00     | 0.00     | 0.04     | 0.08     | 0.17     | 0.04     | 0.08     | 0.09     | 0.13     |
|       | Cangyuan   | 0.42     | 0.22     | 0.47     | 0.17     | 0.47     | 1.39     | 0.19     | 0.31     | 0.20     | 0.17     | 0.00     | 0.08     |
|       | Yingjiang  | 0.11     | 0.31     | 0.45     | 0.06     | 0.17     | 0.31     | 0.86     | 0.50     | 0.36     | 0.03     | 0.00     | 0.03     |
| Hainan | Tianya District | 0.30 | 0.47 | 0.43 | 0.53 | 0.50 | 0.83 | 0.93 | 0.83 | 0.90 | 0.37 | 0.23 |
|       | Wuzhishan | 0.73 | 0.29 | 0.48 | 0.31 | 0.40 | 0.21 |
| Henan | Yongchen | 0.00     | 0.17     | 0.14     | 0.06     | 1.64     | 0.00     | 0.00     | 0.03     | 0.00     | 0.00     | 0.00     |
|       | Shihe     | –       | –       | 2.75     | 9.15     | 3.85     | 6.00     | 2.50     | 1.90     | 2.55     | 1.30     | 0.20     | 0.00     |
|       | Puyang    | 0.00     | 0.00     | 0.17     | 3.03     | 6.03     | 7.31     | 4.75     | 2.59     | 0.72     | 0.50     | 0.00     | 0.00     |
| Guizhou | Congjiang | – | 0.34 | 0.31 | 0.39 | 0.07 | 0.01 |
| Jiangsu | Jintan   | 0.00     | 0.00     | 1.11     | 1.63     | 2.81     | 2.33     | 0.75     | 1.21     | 1.07     | 0.60     | 0.38     | 0.07     |
| Hubei | Zaoyang   | –       | –       | 2.90     | 2.97     | 2.13     | 0.90     | 1.20     | 0.87     | 0.20     | 0.07     | 0.00     | 0.00     |
| Guangdong | Potou | 0.00     | 0.07     | 0.00     | 0.04     | 0.00     | 0.00     | 0.00     | 0.00     | 0.00     | 0.00     | 0.01     | 0.00     |
| Guangxi | Shanglin  | –       | –       | 0.31     | 0.11     | 0.13     | 0.06     | 0.03     |
| Zhejiang | Yiwu     | 0.00     | 0.03     | 0.00     | 0.00     | 0.00     | 0.00     | 0.00     | 0.00     | 0.00     | 0.00     | 0.00     | 0.00     |
| Liaoning | Donggang | 0.00     | 0.00     | 0.00     | 0.00     | 0.05     | 0.16     | 0.45     | 0.00     | 0.00     | 0.00     | 0.00     | 0.00     |

Note: – : Not monitored
Abbreviation: PLAD=provincial-level administrative division.
has gradually increased, making imported malaria a major threat to the elimination of malaria in China (6). Imported malaria cases will exist for a long time after the elimination of malaria in China, a high risk of malaria re-transmission in areas where Anopheles spp. still exist (6). Therefore, continuous surveillance of malaria vectors and analysis of surveillance data are of highest importance for maintaining malaria-free status in China.

The results obtained in this study were consistent with those from previous studies. First, An. sinensis is widely distributed with a high population density and it is still considered of being an important vector for Plasmodium vivax malaria. Except in Xizang (Tibet), the vector, was detected in all surveillance sites, with the highest density in Henan and Anhui provinces. Second, from 1998 to 2001, An. anthropophagus was found in 205 counties of 11 PLADs in China (7). From 2005 to 2010, it was captured in 13 counties in 7 PLADs, including Henan, Hubei, Guangdong, Hainan, Sichuan, Guizhou, and Yunnan (5). However, from 2018 to 2020, it was only detected in Liaoning and Guizhou provinces, indicating that the spatial distribution of An. anthropophagus became smaller than before. Third, mosquitoes of the An. minimus group were recorded from 16 PLADs in southern China, such as Hubei, Henan, Anhui, Zhejiang, Yunnan, Guizhou, Guangxi, and Hainan (5). From 2005 to 2010, An. minimus was captured at 5 surveillance sites in Fujian, Guangdong, Hainan, Guizhou, and Yunnan provinces (5). However, it was detected only in Hainan, Yunnan, and Guangdong provinces during 2018–2020. Fourth, An. dirus was only detected in Hainan Province. In summary, the distribution of An. anthropophagus, An. minimus, and An. dirus fitted well with the areas at risk for malaria re-transmission (8), which provided the references to malaria elimination certification. In contrast, the seasonal distribution patterns of An. sinensis in the 2018–2020 surveillance differed from the previous records that An. sinensis had a classic peak of abundance each year in the rainy season (July–August), while the other malaria vectors had the peak of abundance during August–September in the surveillance sites in 2005–2010 (5).

The study was subject to some limitations. First, the identification level of mosquito species was different in 12 monitoring PLADs. Second, some individual sentinel sites were not monitored due to natural weather conditions, such as that in Xizang (Tibet).

This study reports the first systematic surveillance of malaria vectors in the malaria elimination phase in China, with wide coverage and high representativeness. The surveillance area could be roughly divided into three regions. The southern and southeastern regions of China had high risk for malaria re-transmission and had more detected species of malaria vectors, which may be due to the existence of more suitable breeding sites for Anopheles spp. (5,9). The high density of An. sinensis in central China, located in the climate sensitive zone for malaria transmission, may be related to the high number of malaria cases in the past (9–10). In northeastern region, where climate change is likely to cause an increase in the prevalence of P. vivax in the country, there may be significant risk due to the presence of An. anthropophagus with high ability for malaria transmission. In addition, the surveillance for malaria vectors is from May to October. Due to the increase of global warming, the seasonal fluctuations of Anopheles spp. and their density in some southern, southeastern, and central region sentinel sites cannot be fully reflected. Therefore, it is necessary to frequently carry out standardized and comprehensive surveillance of malaria vectors. According to monitoring results, different areas are classified with different risk levels for malaria re-transmission, and then different targeted prevention and control strategy can be formulated and combined with the surveillance results of imported malaria cases.

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Epidemics of emerging and neglected infectious diseases are severe threats to public health and are largely driven by the promotion of globalization and by international multi-border cooperation. Mosquito-borne viruses are among the most important agents of these diseases, with an associated mortality of over one million people worldwide. The well-known mosquito-borne diseases (MBDs) with global scale include malaria, dengue fever, chikungunya, and West Nile fever, which are the largest contributor to the disease burden. However, the morbidity of some MBDs has sharply decreased due to expanded programs on immunization and more efficient control strategies (e.g., for Japanese encephalitis and yellow fever). Nevertheless, the global distribution and burden of dengue fever, chikungunya, and Zika fever are expanding and growing. Similar to the seemingly interminable coronavirus disease 2019 (COVID-19) pandemic sweeping across the globe since the end of 2020, MBDs could also spread at an unexpected rate and cause great economic damage.

**THE PREVALENCE OF EMERGING MOSQUITO-BORNE ZOONOTIC PATHOGENS**

The Zika virus (ZIKV) causes a traditional mosquito-borne enzootic disease and was first identified in rhesus monkeys in Uganda in 1947, subsequently spreading in Africa, Asia, and the Pacific Islands, and expanding to Brazil in May 2015. China seemed successful in keeping the Zika pandemic at bay with only a few imported cases. However, ZIKV was isolated in mosquitoes from Yunnan, Guizhou, and Jiangxi from 2016 to 2018, and 1.8% of healthy individuals in Nanning, China were positive for the ZIKV antibody. This suggests the existence of the natural circulation of ZIKV between mosquitoes and humans in China even before the international public health emergency. The sudden outbreak of egg drop syndrome caused by the Tembusu virus (TMUV) quickly swept the coastal provinces and neighboring regions in 2010, resulting in severe economic loss in the poultry industry. To date, records of TMUV have covered 18 provinces in China, and are mainly comprised of reports from the last decade. Similarly, the Getah virus, which is mainly transmitted between mosquitoes and domestic livestock, has been spreading across China since 2010, with an outbreak on a swine farm in Hunan in 2017. Moreover, despite having a relatively short history (first detected in 1997), the Liao ning virus (LNV) has been recorded in most of Northern China, including Beijing. It was initially thought that the virus was specific to China, until the virus was isolated from 4 genera of mosquitoes collected along coastal regions of Australia during 1988 to 2014, with a characteristic insect-specific phenotype. By contrast, the Chinese isolates can be replicated in mammalian cell lines and cause viremia and massive hemorrhage during re-infection of mice.

**THE EXTENSIVE GEOGRAPHIC DISTRIBUTION AND THE DIVERSITY OF ISFVs**

Aside from the mosquito-borne zoonotic and potentially pathogenic viruses, the increasing discovery of insect-specific flaviviruses (ISFVs) in the last decade is also worthy of attention. ISFVs, which are specific to insects, have both horizontal and vertical transmission routes, have diverse host relationships, and have a wide geographic distribution. This group can be divided into monophyletic classical ISFVs (cISFVs) and dual-host ISFVs (dISFVs), with the latter being more closely related to mosquito-borne pathogenic flaviviruses (MBPFVs) speculated to have lost their ability to infect vertebrate cells during their
There are three common cISFVs hosted by medically important mosquitoes: the Culex flavivirus (CxFV), the Quang Binh virus (QBV), and the Aedes flavivirus (AeFV) (10, 15). The distribution and host range of the Hanko virus (Inner Mongolia, 2018), the Yunnan Culex flavivirus (Yunnan, 2009; 2018), the Culex theileri flavivirus (Yunnan, 2018), and the Yamadai flavivirus (Yunnan, 2018) in China are relatively localized (10, 16). In some instances, a high prevalence of ISFVs have been observed in the field, such as QBV (21.53/1000) in Cx. pipiens in Jining, CxFV (61.25/1000) in Cx. tritaeniorhynchus in Sanya, and AeFV (33.93/1000) in Aedes albopictus in Songjiang District, Shanghai (10, 15). By contrast, the distribution and host range of dISFVs are narrower than that of cISFVs. The two dISFVs recorded in China, the Chaoyang virus (Liaoning, 2008; Inner Mongolia, 2018) and the Donggang virus (Liaoning, 2009, unpublished in China), are transmitted by Ae. vexans and Cx. pipiens and by Aedes mosquitoes, respectively.

**TABLE 1. Vectors, hosts, geographic distributions, and collection years of emerging mosquito-associated viruses in China (2010–2020).**

| Virus   | Vector       | Vertebrate host | Distribution (year)                              |
|---------|--------------|-----------------|-------------------------------------------------|
| ZIKV    | Armigeres subalbatus  
           Culex quinquefasciatus  
           Cx. tritaeniorhynchus  
           Anopheles sinensis       | Human            | Guizhou (2016); Jiangxi (2018); Yunnan (2016) |
| TMUV    | Cx. pipiens  
           Cx. tritaeniorhynchus  
           Cx. annulus           | Duck             | Anhui (2013); Beijing (2010); Chongqing (2013); Fujian (2010); Guangdong (2011–2015); Guangxi (2011); Hebei (2010); Henan (2010); Hubei (2018); Inner Mongolia (2017); Jiangsu (2010, 2012); Jiangxi (2010); Shanghai (2010, 2011, 2012, 2016); Shanghai (2010); Zhejiang (2010–2016); Yunnan (2012); Sichuan (2013); Taiwan (2019) |
|         | Ae. flavidorsalis  
           Ae. caspius  
           Cx. pipiens  
           Cx. modestus  
           Ae. dorsali  
           Ae. vexans  
           Cx. tritaeniorhynchus  
           Ar. subalbatus  
           Cx. pseudovishnui  
           Cx. fuscocephala  
           Cx. annulus  
           An. sinensis  
           Cx. pipiens       | Mice             | Beijing (2014); Gansu (2011); Jilin (1997); Liaoning (2012); Qinghai (2007); Shanxi (2007); Xinjiang (2005, 2006–2008, 2011) |
| LNV     | Aedes flavidorsalis  
           Ae. caspius  
           Cx. pipiens  
           Cx. modestus  
           Ae. dorsali  
           Ae. vexans  
           Cx. tritaeniorhynchus  
           Ar. subalbatus  
           Cx. pseudovishnui  
           Cx. fuscocephala  
           Cx. annulus  
           An. sinensis  
           Cx. pipiens       | Horse            | Anhui (2017); Gansu (2006); Guangdong (2018); Guizhou (2008); Hainan (1964, 2018); Hebei (2002); Henan (2011); Hubei (2010); Hunan (2017); Inner Mongolia (2018); Jilin (2017, 2018); Liaoning (2006); Shandong (2017); Shanghai (2005); Shanxi (2012); Sichuan (2012, 2018); Taiwan (2002); Yunnan (2005, 2007, 2010, 2012) |
|         | Ae. flavidorsalis  
           Ae. caspius  
           Cx. pipiens  
           Cx. modestus  
           Ae. dorsali  
           Ae. vexans  
           Cx. tritaeniorhynchus  
           Ar. subalbatus  
           Cx. pseudovishnui  
           Cx. fuscocephala  
           Cx. annulus  
           An. sinensis  
           Cx. pipiens       | Swine             | Gansu (2011); Henan (2004); Hubei (2018); Inner Mongolia (2018); Liaoning (2011); Shaanxi (2012); Shandong (2009, 2012, 2018); Shanghai (2016, 2018); Shanxi (2012); Taiwan (2010); Xinjiang (2012) |
|         | Ae. flavidorsalis  
           Ae. caspius  
           Cx. pipiens  
           Cx. modestus  
           Ae. dorsali  
           Ae. vexans  
           Cx. tritaeniorhynchus  
           Ar. subalbatus  
           Cx. pseudovishnui  
           Cx. fuscocephala  
           Cx. annulus  
           An. sinensis  
           Cx. pipiens       | Cattle            | |
|         | Ae. flavidorsalis  
           Ae. caspius  
           Cx. pipiens  
           Cx. modestus  
           Ae. dorsali  
           Ae. vexans  
           Cx. tritaeniorhynchus  
           Ar. subalbatus  
           Cx. pseudovishnui  
           Cx. fuscocephala  
           Cx. annulus  
           An. sinensis  
           Cx. pipiens       | Blue fox         | |
| GETV    | Ae. vexans  
           Cx. pipiens       | Horse            | Hainan (2018); Hebei (2018); Inner Mongolia (2018); Shandong (2018); Shanghai (2016, 2018) |
| CHAOV   | Ae. vexans  
           Cx. pipiens       | Mice             | Liaoning (2008); Inner Mongolia (2018) |
| AeFV    | Ae. albopictus       |                 | Hubei (2018); Shanghai (2016); Yunnan (2018) |
| CxFV    | Cx. pipiens  
           Cx. tritaeniorhynchus  
           An. sinensis  
           Cx. modestus  
           Ae. vexans  
           Cx. tritaeniorhynchus  
           An. sinensis  
           Cx. modestus  
           Ae. vexans       |                 | Gansu (2011); Henan (2004); Hubei (2018); Inner Mongolia (2018); Liaoning (2011); Shaanxi (2012); Shandong (2009, 2012, 2018); Shanghai (2016, 2018); Shanxi (2012); Taiwan (2010); Xinjiang (2012) |
| QBV     | Cx. pipiens  
           Cx. tritaeniorhynchus  
           An. sinensis  
           Cx. modestus  
           Ae. vexans       |                 | Hainan (2018); Hebei (2018); Inner Mongolia (2018); Shandong (2018); Shanghai (2016, 2018) |

Abbreviations: AeFV=Aedes flavivirus; CHAOV=Chaoyang virus; CxFV=Culex flavivirus; GETV=Getah virus; LNV=Liao ning virus; QBV=Quang Binh flavivirus; TMUV=Tembusu virus; ZIKV=Zika virus.

**THE POTENTIAL OF ISFVS AS A NOVEL INTERVENTIONAL TOOL FOR VECTOR CONTROL**

Since vaccines for the majority of MBVs are unavailable, vector control is the major route for routine control and epidemic disposal. However, the intensive use of insecticides in agriculture and pest management has resulted in the development and increase of insecticide resistance in mosquitoes. Therefore, it is urgent to develop novel control strategies and tools. Biological control is the traditional research hotspot, as it is sustainable and environmentally friendly. Bacteria (Bacillus thuringiensis, Wolbachia) have been wildly used in the field. By contrast, the use of fungi (Metarhizium anisopliae and Beauveria bassiana) and viruses (Densovirus) as alternative mosquito control agents remains at the laboratory or semi-field stages. Further studies on ISFVs have led to the discovery of their...
natural, physical, and ecological characteristics, as well as their phylogenetic status, and these clues indicate the potential of ISFVs as a novel interventional tool for vector control, most likely based on the mechanism of superinfection exclusion (17). Moreover, because of their phylogenetic similarity, it seems that dISFVs have a greater potential to inhibit the replication of MBPFVs than cISFVs. Superinfection exclusion can occur between closely related viruses; however, more distantly related viruses do not generally interfere with each other (18). In practice, infection with cISFV and CxFV may reportedly increase the West Nile virus (WNV) infection rate, possibly through facilitation of secondary infections with similar agents by the reduction of immune recognition (18), and because prior infection with cell-fusing agent viruses may reduce the dissemination titer of ZIKV and dengue virus (DENV) both in vitro and in vivo. Other studies have also shown that during instances of prior infection with dISFV, the Nhumirim virus will suppress subsequent replication of mosquito-borne flaviviruses associated with human diseases, including WNV (19), ZIKV, and DENV (20). Nevertheless, further studies are necessary to help us arrive at a consensus regarding whether or not the presence of ISFVs can interfere with infection by MBPFVs, which could also subsequently alter the transmission capacity of certain vector populations for several vector-borne diseases. It is also important to more thoroughly analyze the maintenance cycle of ISFVs and how they escape the host immune system. Furthermore, we should pay more attention to how ISFVs are apparently unable to affect the health of birds, domestic animals, and humans. It is noteworthy that these viruses are carried by medically important mosquitoes and likely to attack vertebrate immune

FIGURE 1. Geographic distribution and diversity of insect specific flaviviruses in China by 2020. Abbreviations: AAFV=Anopheles associated flavivirus; AeFV=Aedes flavivirus; CHAOV=Chaoyang virus; CTFV=Culex theileri flavivirus; CxFV=Culex flavivirus; HANKV=Hanko virus; QBV=Quang Binh flavivirus; YDFV=Yamada flavivirus; YNCxFV=Yunnan Culex flavivirus.
system when vertebrate innate immunity pathways are disabled by known pathogenic flaviviruses (21), which represent a potential threat to both human and animal health.

THE CHALLENGES OF MBV CONTROL DURING THE COVID-19 PANDEMIC

Emerging and preexisting MBVs are spreading globally at an unexpected rate. MBD surveillance may have been constrained by the COVID-19 pandemic, which has drawn the most attention with regards to public health, but hampers the expansion of MBVs because of restrictions in international travel. Routine mosquito surveillance and screening for mosquito-borne pathogens can be early indicators for local disease transmission and outbreaks. These practices also highlight that wide-ranging, systematic, and continuous molecular monitoring of mosquito-borne circulating viruses in vectors is urgently needed. This monitoring would provide a comprehensive understanding of virus diversity, geographic distribution, evolution, shifts in circulating genotypes, and infection rates in China and other neighboring countries and allow accurate and timely estimations of the true disease burden and prevalence of emerging/re-emerging and known mosquito-borne pathogens. This is essential to support the decision-making process regarding appropriate prevention and control strategies in China, neighboring countries, and countries involved in the Belt and Road Initiatives. Moreover, a close watch on the dynamics of mosquito insecticide resistance, alternative insecticides in certain areas, and the proper use of insect growth regulators or biocontrol approaches for integrated vector control programs should also be considered to mitigate and slow the spread and impact of insecticide resistance development in disease vector populations. The biodiversity, widespread presence, and variety of mosquito host species of ISFVs in nature shed light on means of indirect protection against the dissemination of MBVs. Ultimately, there is also an urgent need to develop an MBV vaccine using strains that are prevalent in the field to reduce the increasing health risks posed by MBVs.

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SUPPLEMENTARY TABLE S1. Vectors, hosts, geographic distributions, and collection years of emerging mosquito-associated viruses in China (2010–2020).

| Virus | Vector | Vertebrate host | Distribution (year) |
|-------|--------|-----------------|---------------------|
| ZIKV  | Armigeres subalbatus | Human | Guizhou (2016) (1) |
|       | Culex quinquefasciatus | | Yunnan (2016) (2) |
|       | Cx. tritaeniorhynchus | | Jiangxi (2018) (3) |
|       | Anopheles sinensis | | |
|       | | | |
|       | Cx. pipiens | Duck | Anhui (2013) (4) |
| TMUV  | Cx. tritaeniorhynchus | Goose | Beijing (2010) (5) |
|       | Cx. annulus | Chicken | Chongqing (2013) (6) |
|       | | Sparrow | Fujian (2010) (7) |
|       | | Pigeon | Guangdong (2011–2015) (8) |
|       | | | Guangxi (2011) (9) |
|       | | | Hebei (2010) (5) |
| LNV   | Ae. flavidorsalis | Mice | Henan (2010) (9) |
|       | Ae. caspius | | Hubei (2018) (8) |
|       | Cx. pipiens | | Inner Mongolia (2017) (4) |
|       | Cx. modestus | | Jiangsu (2010, 2012) (5,9) |
|       | Ae. dorsali | | Jiangxi (2010) (5) |
|       | Ae. vexans | | Shandong (2010, 2011, 2012, 2016) (4–5,9–10) |
|       | | | Shanghai (2010) (11) |
|       | | | Zhejiang (2010–2016) (10,12) |
|       | | | Yunnan (2012) (13) |
|       | | | Sichuan (2013) (6) |
|       | | | Taiwan (2019) (14) |
|       | | | |
| GETV  | Cx. tritaeniorhynchus | Horse | Shanxi (2007) (15) |
|       | Ar. subalbatus | Swine | Beijing (2014) (16) |
|       | Cx. pseudovishnui | Cattle | Gansu (2011) (17) |
|       | Cx. fuscocephala | Blue fox | Jinlin (1997) (18) |
|       | Cx. annulus | | Liaoning (2012) (19) |
|       | An. sinensis | | Qinghai (2007) (20) |
|       | Cx. pipiens | | Xinjiang (2005, 2006–2008, 2011) (21–23) |
|       | | | Jinlin (2017, 2018) (24) |
|       | | | Inner Mongolia (2018) (25–26) |
|       | | | Hubei (2010) (27) |
|       | | | Shanxi (2012) (28) |
|       | | | Gansu (2006) (29) |
|       | | | Shandong (2017) (30) |
|       | | | Henan (2011) (37) |
| CHAOV | Ae. vexans | Horse | Anhui (2017) (GenBank, unpublished data) |
|       | Cx. picipiens | Swine | Hebei (2002) (29) |
|       | | Cattle | Sichuan (2012, 2018) (32–33) |
|       | | | Shanghai (2005) (29) |
|       | | | Guizhou (2008) (34) |
|       | | | Hunan (2017) (35) |
|       | | | Yunnan (2005, 2007, 2010, 2012) (29,34) |
|       | | | Guangdong (2018) (36, 37) |
|       | | | Hainan (1964, 2018) (38) |
|       | | | Taiwan (2002) (39) |
|       | | | Liaoning (2016) (40) |
| AeFV  | Ae. albopictus | | Hubei (2018) (25) |
|       | Cx. picipiens | | Shanghai (2016) (42) |
|       | | | Yunnan (2018) (43) |
|       | | | Liaoning (2011) (44) |
|       | | | Shandong (2009, 2012, 2018) (25,45) |
|       | | | Henan (2004) (45) |
|       | | | Shaanxi (2012) (45) |
|       | | | Shanxi (2012) (46) |
|       | | | Shanghai (2016, 2018) (25,42) |
|       | | | Hubei (2018) (25) |
|       | | | Inner Mongolia (2018) (25) |
|       | | | Gansu (2011) (17) |
|       | | | Taiwan (2010) (47) |
|       | | | Xinjiang (2012) (48) |
### TABLE S1. (Continued)

| Virus | Vector | Vertebrate host | Distribution (year) |
|-------|--------|-----------------|---------------------|
| QBV   | Cx. tritaeniorhynchus | Shanghai (2016, 2018) (25, 42) |
|       | Cx. piensis | Shandong (2018) (25) |
|       | An. sinensis | Inner Mongolia (2018) (25) |
|       |            | Hubei (2018) (25) |
|       |            | Hainan (2018) (25) |

Abbreviations: AeFV=Aedes flavivirus; CHAOV=Chaoyang virus; CxFV=Culex flavivirus; GETV=Getah virus; LNV=Liao ning virus; QBV=Quang Binh flavivirus; TMUV=Tembusu virus; ZKV=Zika virus.

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