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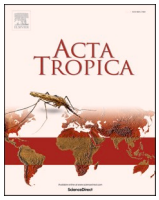

The rising proportion of *Plasmodium ovale* spp. in imported malaria in Anhui Province, China: A retrospective propensity score-matched case-control study

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ABSTRACT

No autochthonous malaria cases have been reported in Anhui Province, China, since 2014. However, imported malaria remains a significant public health concern. Moreover, the proportion of reported imported malaria cases attributed to *Plasmodium ovale* spp. (*P. ovale* spp.) has risen to unexpectedly high levels. The factors contributing to this increase and its potential impact on the healthcare system remain unknown. A retrospective case-control study utilizing surveillance data from 2012 to 2023 was conducted to explore these issues. By comparing individuals infected with *P. ovale* spp. to those with *P. falciparum* and employing propensity score matching, the research assessed epidemiological and clinical data. Results indicated that the proportion of cases from *P. ovale* spp. increased significantly ($\chi^2 = 9.388$, $P = 0.002$), reaching 50.00 % in 2021. Differences between groups were noted in previous infection history ($\chi^2 = 8.358$, $P = 0.004$), overseas stay duration ($\chi^2 = 7.856$, $P = 0.049$), and onset timing ($W = 2991.000$, $P < 0.001$). *P. ovale* spp. cases had longer intervals from symptom onset to first medical visit and diagnosis than *P. falciparum* cases. Initial diagnosis and species identification were less accurate for *P. ovale* spp. (62.99 % and 30.52 %) compared to *P. falciparum* (74.03 % and 93.51 %). The increase in infections can be attributed to repeated exposure, which heightens the risk of contracting *P. ovale* spp. during extended stays in endemic regions, as well as to insufficient treatment of hepatic hypnozoites. It underscores the need for Anhui Province's healthcare facilities to enhance their diagnostic and treatment capacities for *P. ovale* spp., particularly through more sensitive detection techniques.

1. Introduction

Malaria, a disease-causing considerable morbidity and mortality in tropical and subtropical regions, remains a significant global public health challenge. The international community is dedicated to the ambitious goal of eliminating malaria. Progress towards malaria elimination has been observed in a multitude of countries. From 2000 to 2022, 25 countries that were endemic for malaria in 2000 successfully achieved three consecutive years with no indigenous cases, of which twelve countries were officially certified as malaria-free by the World Health Organization (WHO) (World Health Organization, 2024). However, there was a concerning rise in reported malaria cases worldwide in 2022, totaling 249 million, which marks an increase from 232 million cases reported in 2019 (World Health Organization, 2024). This recent rebound has been attributed to disruptions in malaria control efforts caused by the COVID-19 pandemic, raising concerns that this upward trend may have a more pronounced impact due to the growing frequency of global interactions.

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Anhui Province, located in southeastern China, initiated a provincial malaria elimination plan in 2010. No locally transmitted cases have been reported since 2014. In 2019, Anhui Province passed sub-national malaria elimination certification (Xu et al., 2020). In May 2021, Anhui Province, due to its historical receptivity to malaria, underwent evaluation by an independent Malaria Elimination Certification Panel established by the WHO. By 2023, Anhui Province had maintained its malaria-free status for ten years. Nevertheless, with globalization and increased international movement, the province is facing a challenge from imported malaria cases (Li W et al., 2020; Zhang et al., 2019). It is noteworthy that dynamic surveillance data have shown a rising trend in malaria infections attributed to *Plasmodium ovale* spp. (*P. ovale* spp.) in Anhui Province, reaching peaks of nearly 20 % (Zhang et al., 2021). During the COVID-19 pandemic, the proportion of *P. ovale* spp. cases was 50 % (Zhang et al., 2022).

P. ovale spp. was first described in 1922 by Stephens, named for the oval shape of some infected erythrocytes (None, 1914), commonly found in Africa, Southeast Asia, and Papua New Guinea (Okafor CN and Finnigan NA, 2023). Since 2010, *P. ovale* spp. has been divided into two sympatric species, *Plasmodium ovale curtisi* (*P.o. curtisi*) and *Plasmodium ovale wallikeri* (*P.o. wallikeri*), based on gene polymorphisms identified by Sutherland (Sutherland CJ et al., 2010). However, there is ongoing controversy regarding the formal naming of these two distinct parasite forms(Šlapeta J, et al., 2024; Snounou G, et al., 2024). In general, infection due to *P. ovale* spp. has generally been considered rare. According to the World Malaria Report 2023, *P. ovale* spp. infection accounts for less than 1 % of all malaria cases worldwide (World Health Organization, 2024). In France and the United States, *P. ovale* spp. accounts for about 5–6 % of imported malaria cases (Joste V et al., 2021; Mace KE et al., 2022). Therefore, a proportion of 5 % of imported malaria cases attributable to *P. ovale* spp. may be considered a reference value. This study aims to explore the factors contributing to the rising proportion of *P. ovale* spp. in imported malaria cases in Anhui Province and assess its implications for the healthcare system.

2. Methods

2.1. Study design

Malaria is designated as a notifiable infectious disease in China, requiring the reporting of each suspected case(Wang L et al., 2008). Individuals exhibiting either typical or atypical symptoms of malaria, combined with a history of travel to malaria-endemic regions, were classified as suspected cases, irrespective of laboratory evidence. Using routine surveillance data from 2012 to 2023, we conducted a propensity case–control study. During the study period, all infections attributed to *P. ovale* spp. (single infection) were included in the case group. Propensity score matching (PSM) was utilized to select a comparable control group from individuals infected with *P. falciparum* (single infection).

2.2. Data collection

Anhui Province operates a malaria surveillance system consisting of passive and active case detection. When a suspected malaria case is identified, it must be reported to the Centers for Disease Control and Prevention (CDC) through the China Information System for Disease Control and Prevention (CISDCP). Subsequently, professionals from the county-level CDC carry out an epidemiological investigation using a standardized questionnaire. The collected data is then entered into the Information System for Parasitic Disease Control and Prevention (ISPDCP), which is a component of the CISDCP. Additionally, an epidemiological report is generated to document the critical findings of the investigation. In this study, epidemiological and clinical data on malaria cases were extracted from the CISDCP, ISPDCP, and epidemiological survey reports. This data includes demographic information, travel history, clinical symptoms, treatment, and diagnostic outcomes.

2.3. Case confirmation

In the study, whole blood samples were collected from patients before antimalarial treatment by staff at the county-level CDC and subsequently sent to the Malaria Diagnostic Reference Laboratory of Anhui Province within three days. Appendix 1 illustrates the standard operational flow of the reference laboratory. At the reference laboratory, DNA was extracted using the QIAamp DNA Mini kit (QIAGEN Inc, Hilden, Germany) following the manufacturer's instructions. The final diagnosis was confirmed by examining Giemsa-stained thick and thin blood films under an oil immersion lens at 1000 × magnification, and/or through real-time Polymerase Chain Reaction (PCR) analysis. The reaction conditions adhered to the manufacturer's recommendations. Commercial real-time PCR kits (Shanghai ZJ Bio-tech Co., Ltd, Shanghai, China), designed based on a previous study (Perandin F et al., 2004), were utilized to differentiate *Plasmodium* species. The detection limit of the reagent kit is 1×10^3 copies/mL. If *P. ovale* spp. was detected during the species identification process, commercial real-time PCR kits (Shanghai BioGerm Medical Biotechnology Co., Ltd) were utilized. These kits, designed based on a previous study (Bauffe F et al., 2012), were used to differentiate between *P.o. curtisi* and *P.o. wallikeri,* with a detection limit of 1×10^3 copies/mL. The outcomes of co-infections were verified through PCR detection. Severe malaria was defined according to the clinical criteria outlined in "Diagnosis of Malaria" (People's Republic of China Health Industry Standard, WS 259–2015). These criteria include a confirmed case accompanied by one or more of the following features: impaired consciousness or coma, severe anemia (hemoglobin < 5 g/dL, hematocrit < 15 %), renal impairment (serum creatinine > 265 μmol/L), pulmonary edema or acute respiratory distress syndrome, hypoglycemia (< 2.2 mmol/L or < 40 mg/dL), circulatory collapse or shock (systolic blood pressure < 70 mm Hg in adults and < 50 mm Hg in children), and metabolic acidosis (plasma bicarbonate < 15 mmol/L). Imported cases are defined as malaria cases where the infection was acquired outside the country of diagnosis. (World Health Organization, 2021)

2.4. Statistical analysis

In the study, all statistical analyses were conducted using R software (Version 4.2.2), MSTATA software (www.mstata.com), and JASP (Version 0.18.3). MapInfo (Version 15.0) was employed to generate the thematic map. To control potential confounders, we utilized Propensity Score Matching (PSM) to match individuals in the case group (infections with *P. ovale* spp.) with those in the control group (infections with *P. falciparum*). Optimal pair matching was performed. Descriptive and comparative statistics were used to present the epidemiological profile. Normally distributed continuous variables were described using the mean and standard deviation (SD), while non-normally distributed variables were presented as the median and interquartile range (IQR). The Mann–Whitney U test was employed for comparing non-normally distributed variables. Categorical data were summarized as numbers and percentages, and differences in proportions were assessed using appropriate statistical tests such as Pearson's chi-squared (χ^2) test or Fisher's exact test. The chi-squared test for linear trend was applied to detect significant time trends. For statistical significance, a one-sided or two-sided P value less than 0.05 was considered, dependent on the specific conditions.

3. Results

3.1. Propensity score matching

The study considered variables such as age, travel destination, gender, and occupation as potential confounding factors between the case group (*P. ovale* spp., excluding cases of co-infection) and the control group (*P. falciparum*, excluding cases of co-infection). Following the

application of 1:1 PSM, all Standardized Mean Difference (SMD) values were below 0.01, indicating comparable baseline characteristics across the two groups. Additional details can be found in Table 1. Post PSM, there were 154 individuals in each of the cases and control groups.

3.2. Epidemiological profile

Between 2012 and 2023 in Anhui Province, a total of 1086 imported malaria cases were reported, with 98.25 % originating from Africa. Among these cases, *P. falciparum* was the predominant species, representing 833 cases (76.70 %), followed by *P. ovale* spp. (154 cases, 14.18 %), *P. malariae* (44 cases, 4.05 %), *P. vivax* (47 cases, 4.33 %), and co-infection (8 cases, 0.74 %). Among 8 cases of co-infection, there were 6 cases of *P. falciparum* co-infected with *P. ovale* spp., one of *P. falciparum* with *P. malariae*, and one of *P. malariae* with *P. ovale* spp.. The proportion of imported cases attributed to *P. ovale* spp. (excluding co-infections) showed a rising trend ($\chi^2 = 9.388$, $P = 0.002$), reaching a peak of 50.00 % in the year 2021. (Fig. 1). Out of the 154 patients infected with *P. ovale* spp., 153 had single-species infections (88 *P. o. curtisi* and 65 *P. o. wallikeri*), while one case showed a co-infection of *P. o. curtisi* and *P. o. wallikeri*. All 161 patients infected with *P. ovale* spp., including 7 co-infection cases, were imported from 17 African countries and 1 asian country (Philippines). The primary sources of these infections were Equatorial Guinea (25 cases, 15.53 %), Angola (24 cases, 14.91 %), Nigeria (21 cases, 13.04 %), Democratic Republic of the Congo (18 cases, 11.18 %), and Cameroon (15 cases, 9.32 %). Both *P. o. curtisi* and *P. o. wallikeri* were concurrently identified in 13 of the 18 countries from which cases were imported (Fig. 2). *P. ovale* spp. and *P. falciparum* exhibit statistically significant differences in terms of the previous history of malaria infection, duration of overseas stay, and days from return and the onset of illness (Table 2). The median time interval from return to onset of *P. o. curtisi* was 76.5 (24.75,203) days, significantly longer than that of *P. o. wallikeri*, which was 31 (12,101) days ($W = 3664.000$, $P = 0.003$). In cases of *P. ovale* spp. without a previous history of malaria infection, the median time interval from return to onset of *P. o. curtisi* and *P. o. wallikeri* was 89.5 (20, 204.5) days and 32 (10, 206) days. There was no significant statistical difference detected between the two groups ($W = 150.000$, $P = 0.472$).

3.3. Clinical manifestations

Among the *P. ovale* spp. group, common clinical symptoms include fever (94.16 %), chills (85.71 %), sweating (63.64 %), and headaches (54.55 %), similar to those seen in the *P. falciparum* group. The *P. ovale* spp. group did not report any severe cases, whereas 9 cases of severe malaria (5.84 %) were observed in the *P. falciparum* group. Additional clinical feature information is presented in Table 3.

3.4. Diagnosis and treatment process

This section elaborates on the diagnosis and treatment process of *P. ovale* spp. and *P. falciparum* and contrasts their distinguishing characteristics. The median time intervals in the *P. ovale* spp. group, from symptom onset to the first medical visit, from the first visit to malaria diagnosis, and from symptom onset to diagnosis, were 1 (0, 3) day, 1 (0, 3) day, and 3 (1, 5.75) days, respectively. And the median time intervals in the *P. falciparum* Group were 1 (0, 2) day, 0 (0, 1) day, and 2 (1, 3.75) days. Significant statistical differences were observed in all time intervals: from symptom onset to the first medical visit ($W = 10{,}405.000$, $P = 0.027$), from the first visit to malaria diagnosis ($W = 9855.500$, $P = 0.003$), and from symptom onset to diagnosis ($W = 9287.000$, $P < 0.001$). These intervals were longer than those observed in the *P. falciparum* group. No statistical disparity was observed in the selection of healthcare facilities when patients from both the case and control groups sought medical attention for the first time ($\chi^2 = 7.708$, $P = 0.173$). The *P. falciparum* group exhibited higher accuracy rates in initial diagnosis and species identification, at 74.03 % and 93.51 %, respectively, compared to 62.99 % and 30.52 % in the *P. ovale* spp. group. In *P. ovale* spp. cases, primaquine is recommended after a definitive diagnosis. Out of 154 cases of *P. ovale* spp., five (3.25 %) experienced a relapse post-treatment. The intervals between the replase of the 5 cases were 39 days, 39 days, 48 days, 243 days, and 400 days, respectively. 154 cases of *P. ovale* spp., five (3.25 %) experienced a relapse post-treatment. The intervals between the replase of the 5 cases were 39 days, 39 days, 48 days, 243 days, and 400 days, respectively. Among the five relapses, four were attributed to *P. o. curtisi* and one to *P. o. wallikeri* (Table 4).

In the *P. ovale* spp. group, only 30.52 % of cases had accurate species identification. Misdiagnoses occurred, with 27.27 % identified as *P. vivax*, and 8.44 % as *P. falciparum*. In the remaining 33.77 % of cases, species identification was not conducted, and only the parasite was identified. Conversely, the species identification for the *P. falciparum* group yielded accurate results in 93.51 % of cases. Additional details of the diagnostic results are presented in Table 5.

3.5. Epidemiological response

In China, the "1–3–7" surveillance and response approach is implemented and monitored for the management of malaria cases. Specifically, this strategy aims to report cases within 1 day of diagnosis, conduct complete case investigations within 3 days of reporting, and finalize foci investigation and response within 7 days (Cao J et al., 2014). The *P. ovale* spp. group achieved a 100.00 % reporting rate within 1 day, a 94.1 % completion rate for epidemiological investigations within 3 days, and a 92.8 % disposal rate of foci within 7 days. Similarly,

Table 1
Baseline characteristics of case and control groups before and after propensity score matching.

Variables	Before PSM			After PSM		
	P. falciparum ($n = 833$)	*P. ovale* spp.($n = 154$)	SMD[a]	*P. falciparum* ($n = 154$)	*P. ovale* spp. ($n = 154$)	SMD[a]
Age(mean ± SD)	41.03 ± 8.94	43.44 ± 8.96	0.270	43.48 ± 9.19	43.44 ± 8.96	−0.004
Destination n (%)						
West Africa	226(27.1)	43(27.9)	0.018	48(31.2)	43(27.9)	−0.072
Central Africa	300(36.0)	71(46.1)	0.202	67(43.5)	71(46.1)	0.052
South Africa	262(31.5)	35(22.7)	−0.208	35(22.7)	35(22.7)	0.000
East Africa	39(4.7)	4(2.6)	−0.131	4(2.6)	4(2.6)	0.000
Southeast Asia	6(0.7)	1(0.6)	−0.009	0(0.0)	1(0.6)	0.081
Sex n (%)						
Male	817(98.1)	153(99.4)	0.158	154(100.0)	153(99.4)	−0.081
Female	16(1.9)	1(0.6)	−0.158	0(0.0)	1(0.6)	0.081
Occupation n (%)						
Worker	738(88.6)	139(90.3)	0.056	141(91.6)	139(90.3)	−0.044
Waiter	68(8.2)	12(7.8)	−0.014	9(5.8)	12(7.8)	0.073
Other	27(3.2)	3(1.9)	−0.094	4(2.6)	3(1.9)	−0.047

[a] : Standardized Mean Difference

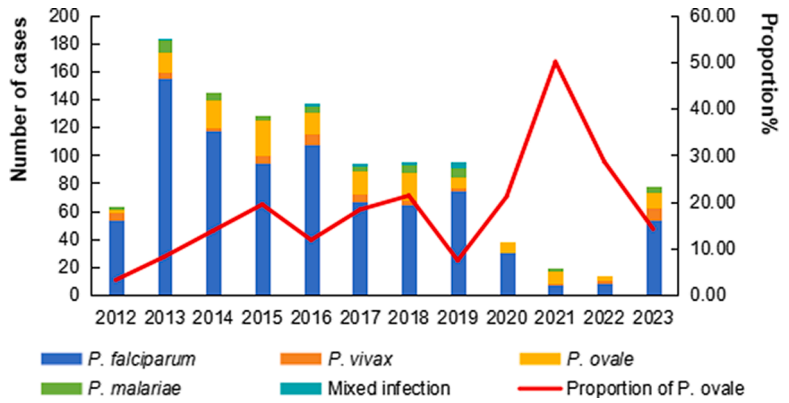

Fig. 1. Imported malaria in Anhui Province, 2012–2023.

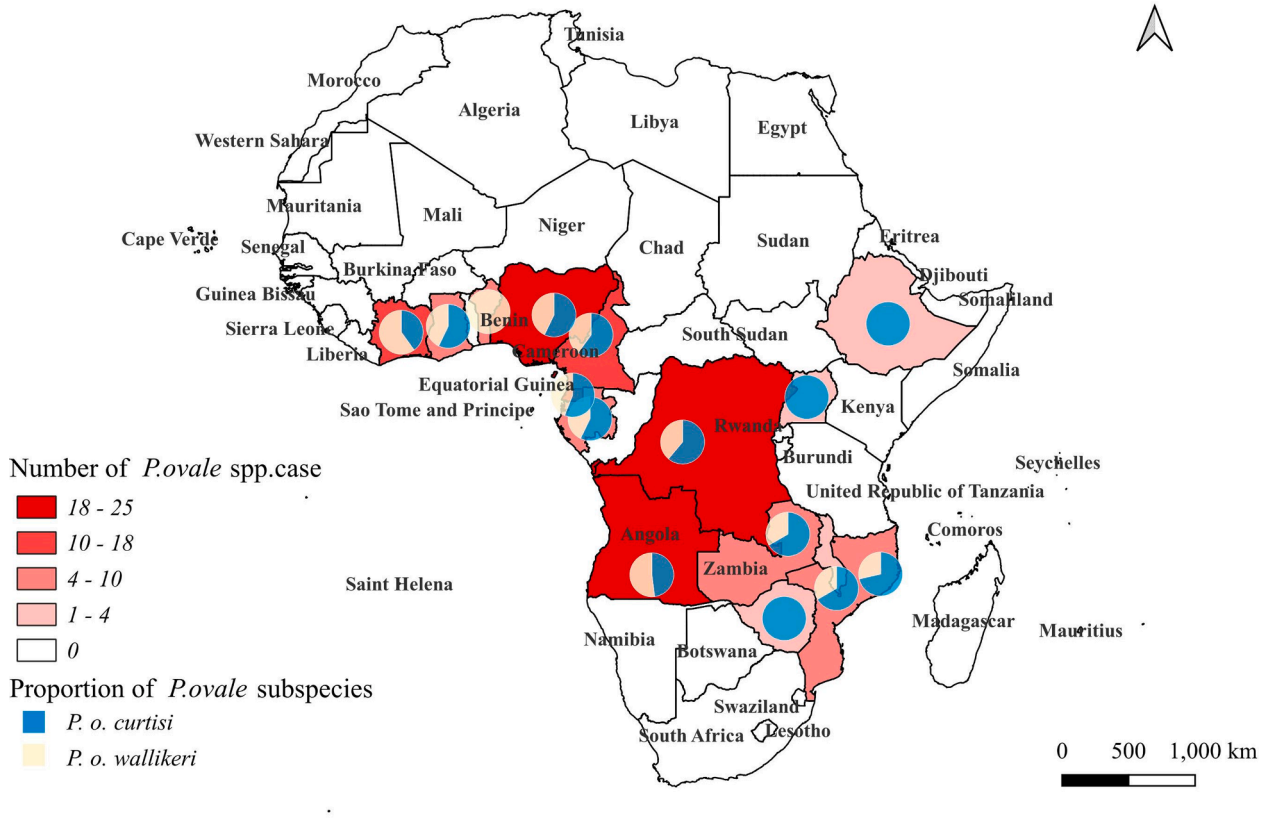

Fig. 2. Geographic distribution of the origin of imported cases of *P. ovale* spp. in Anhui Province, 2012–2023.

the *P. falciparum* group demonstrated rates of 100.00 %, 95.4 % and 96.7 % for the same indicators, respectively. Statistical analysis did not reveal any significant differences between the performance of the two groups.

4. Discussion

Preventing the re-establishment of malaria is currently a key focus in Anhui Province. An adaptive framework has been methodically devised and implemented to tackle this critical issue (Zhang et al., 2024). Surveillance holds a crucial role within this framework. Based on the monitoring results, the percentage of reported malaria cases attributed to *P. ovale* spp. has increased beyond anticipated levels. This study seeks to elucidate the underlying driving forces of this phenomenon and assess its implications.

Discovered by Stevens more than a century ago, *P. ovale* spp. are predominantly found in sub-Saharan Africa (Collins WE and Jeffery GM, 2005). Medical research on this species of *Plasmodium* has been limited. Nevertheless, emerging evidence suggests that the significance of *P. ovale* spp. may have been underestimated (Dinko B et al., 2013; Groger M et al., 2018; Okafor CN and Finnigan NA, 2023). In cross-sectional surveys conducted in Uganda and Equatorial Guinea the findings indicate that *P. ovale* spp. infections are more prevalent than previously estimated, with a frequency ranging from 1 % to 6 % (Oguike MC et al., 2011). In the Democratic Republic of Congo Among, the incidence of *P. ovale* spp. infections within one year were 4.8 % (95 % CI: 3.7 % - 5.9 %) (Sendor R et al., 2023). The highest prevalence of *P. ovale* spp. has been reported in Papua New Guinea and Nigeria, reaching approximately 15 % (May J et al., 1999; Mehlotra RK et al.,2000). Significant proportions of the *P. ovale* spp. have also been identified in Asia, as demonstrated by German returnees from tropical regions (Frickmann H et al., 2019). In Anhui Province, most cases of *P. ovale* spp. are imported from Africa. These findings align with the literature cited

Table 2
Comparison of epidemiological characteristics between the case group and the control group.

Variables	*P. ovale* spp. ($n = 154$)	*P. falciparum* ($n = 154$)	χ^2/W	*P* value
Previous malaria, N (%)				
Yes	121 (78.57)	98 (63.64)	8.358	0.004
No	33(21.43)	56(36.36)		
Duration of overseas Stay (days), N (%)[a]				
≤ 30	3 (1.99)	6 (3.95)	7.856	0.049
31∼	20(13.25)	31 (20.40)		
181∼	30 (19.87)	40 (26.32)		
> 365	98 (64.90)	75 (49.34)		
Days from return to onset, median (IQR)	61(17, 169)	6(2,12)	2991.000	<0.001

[a] : The durations of overseas stays were not available for 3 cases of *P. ovale* spp. and 2 cases of *P. falciparum*.

The matching factors used in PSM are not displayed in the table.

Table 3
Clinical manifestations of case and control groups.

Clinical manifestation	*P. ovale* spp. ($n = 154$)	*P. falciparum* ($n = 154$)	χ^2	*P* value
Fever, N (%)	145(94.16)	140(90.91)	1.175	0.278
Chills, N (%)	132(85.71)	121(78.57)	2.678	0.102
Sweating, N (%)	98(63.64)	86(55.84)	1.944	0.163
Headaches, N (%)	84(54.55)	94(61.04)	1.331	0.249
Diarrhea, N (%)	10(6.49)	15(9.74)	1.088	0.297
Severe[a]**, N (%)**[b]	0(0.00)	9(5.84)	NA	<0.001

[a] : severe malaria is defined according to the guidelines established by the National Health and Family Planning Commission of China(National Health and Family Planning Commission of the People's Republic of China, 2015).
[b] : differences in proportions were tested by Fisher's exact test
NA: not applicable.

Table 4
The diagnosis and treatment process of case and control groups.

Variables	*P. ovale* spp. ($n = 154$)	*P. falciparum* ($n = 154$)	χ^2/W	*P* value
Days from onset to first medical visit, median (IQR)[a]	1(0,3)	1(0,2)	10,405.000	0.027
Days from first medical visit to diagnosis, median (IQR)[a]	1(0,3)	0(0,1)	9855.500	0.003
Days fromonset to diagnosis, median (IQR)[a]	3(1,5.75)	2(1,3.75)	9287.000	<0.001
The level of health facilities for initial medical visit, N (%)				
Private and village clinics	30(19.48)	21(13.64)	7.708	0.173
Primary hospitals	11(7.14)	8(5.20)		
County-level hopsitals	54(35.07)	46(29.87)		
City-level hospitals	32(20.78)	47(30.52)		
Provincial hospitals	27(17.53)	30(19.48)		
Other	0(0.00)	2 (1.30)		
The accuracy of the initial diagnosis, N (%)	97(62.99)	114(74.03)	4.349	0.037
The rate of parasite detection, N (%)	142 (92.21)	147(95.45)	1.883	0.170
The accuracy of species identification, N (%)	47(30.52)	144(93.51)	129.681	<0.001
Relapse, N (%)	5(3.25)	NA	NA	NA
Recrudescence, N (%)[b]	1(0.65)	7(4.55)	NA	<0.001

[a] : a one-sided test was conducted to examine the hypothesis that *P. falciparum* < *P. ovale* spp.
[b] : differences in proportions were tested by Fisher's exact test.
NA: not applicable.

Table 5
The diagnostic results of case and control groups in healthcare facilities.

Species	N	The diagnostic results of healthcare facilities			
		P. falciparum	*P. ovale* spp.	*P.vivax*	Unclassified
***P. falciparum*,N (%)**	154	144(93.51)	1(0.65)	0(0.00)	9(5.84)
***P. ovale* spp., N (%)**	154	13(8.44)	47(30.52)	42 (27.27)	52(33.77)

earlier, providing further evidence for the underestimated prevalence of *P. ovale* spp.. However, this rationale may not sufficiently explain instances in which *P. ovale* spp. account for up to 20 % of the total imported malaria cases in some years in Anhui Province. The peak of 50 % in 2021 was attributed to the implementation of a 14-day quarantine period for COVID-19 upon entry to China. This measure facilitated the detection and reporting of *P. falciparum* infection during the quarantine period at the port of entry (Wu D et al., 2021; Zhang et al., 2022). Anhui is an inland province; therefore, travelers were permitted to return only after completing the quarantine period. During the analysis, we found that *P. ovale* spp. had longer overseas stays and a higher percentage of previous malaria infections compared to those with *P. falciparum*. This implies that individuals may face a higher risk of acquiring *P. ovale* spp. due to repetitive exposure to mosquite bite during their time overseas. On the other hand, *P. ovale* spp. harbor dormant parasites, known as hypnozoites, in the liver (Okafor CN and Finnigan NA, 2023). According to our comprehension, overseas laborers represent the majority of imported malaria cases in Anhui Province. Following malaria contraction overseas, there is limited species identification, potentially leading to the failure to administer targeted treatment with *primaquine* for complete hypnozoite eradication, thereby increasing the risk of *P. ovale* spp. relapse. Based on prior research, relapses were observed to transpire from 17 days post-treatment of the initial attack to as long as 255 days (Chin W and Coatney GR, 1971), and sometimes even beyond. Therefore, individuals may onset due to relapse after returning to China. So, this study proposes that the increased proportion of *P. ovale* spp. in imported malaria cases in Anhui Province may be linked to insufficient treatment for individuals infected with *P. ovale* spp. during overseas. It is important to note that other factors may also influence these findings, including the application of more sensitive nucleic acid testing methods. Further research is necessary to confirm these observations and to deepen our understanding of *P. ovale* spp. and its global impact.

Relapses also serve as a significant confounding factor in determining the latency period of *P. ovale* spp.. Recent studies on imported malaria defined the latency period of *P. ovale* spp. as the duration between departure from a malaria-endemic region and the onset of symptoms in a non-endemic area (Cao Y et al., 2016; Rojo-Marcos G et al., 2018; Xia J et al., 2020; Zhou R et al., 2019). A meta-analysis integrating data from eight prior studies demonstrated that *P.o. curtisi* displayed extended latency periods (Mahittikorn A et al., 2021). In accordance with the definition, our study produced similar results, with *P. o. curtisi* exhibiting a latency period of 89.5 days and *P. o. wallikeri* exhibiting 32 days. It should be noted that distinguishing between a relapse and a primary attack of *P. ovale* spp. requires sufficient epidemiological and laboratory evidence, a task that is frequently difficult to accomplish in field settings. Hence, it is essential to approach the outcomes derived from the aforementioned definition with caution. In this

study, we selected cases without prior malaria infection history for an in-depth analysis of the latency period of *P. ovale* spp. subspecies. However, no positive outcomes were identified.

In addition to investigating the potential contributing factors to the rising proportion of *P. ovale* spp., our primary aim is to promptly detect *P. ovale* spp. cases and deliver appropriate treatment and management. Nevertheless, we are currently encountering several challenges in pursuing this objective. In comparison to *P. falciparum*, the *P. ovale* spp. group exhibits longer intervals from symptom onset to the initial medical consultation and from the first consultation to malaria diagnosis. This suggests that both patient awareness of seeking medical care and doctor's diagnostic awareness are limited in terms of *P. ovale* spp.. It was mainly because that *P. ovale* spp. is a protozoan that causes benign tertian malaria, as it is a slow-growing species and rarely causes severe malaria in humans (Collins WE and Jeffery GM, 2005). Patients frequently delay seeking prompt medical care due to mild symptoms. In addition, the median time from return to onset of *P. ovale* spp. was over a month. Physicians may sometimes neglect to inquire about the patient's long-term epidemiological history. Understanding the characteristic fever cycles of ovale malaria is beneficial for diagnosis in this context. Furthermore, a low parasitemia level in *P. ovale* spp. infection can be missed by the low sensitivity of the microscopic method, presenting a similar challenge for other malaria types. (Okafor CN and Finnigan NA, 2023). At present, the majority of Rapid diagnostic tests (RDTs) products are effective in detecting *P. falciparum* but have a degree of ineffectiveness when detecting *P. ovale* spp. (Tang J et al., 2019). These factors can lead to delays in seeking medical care and diagnosis, contributing to the link with severe malaria (Lu G et al., 2022; Zhang et al., 2022). The most recent systematic review reported that 3 % of *P. ovale* spp. malaria cases developed severe complications according to the WHO 2015 guideline (Groger M et al., 2017). Fortunately, during the study period, no emergency incidents attributed to *P. ovale* spp. were reported. In contrast to a severity rate of 5.84 % in *P. falciparum*, the clinical manifestation of *P. ovale* spp. tends to be milder, primarily due to its low parasitemia levels. While cases of *P. ovale* spp. generally have a favorable prognosis, five cases (3.25 %) documented replase events. In the *P. ovale* spp. group, the species identification rate was merely 30.52 %, whereas it was significantly higher at 93.51 % for *P. falciparum*. This clear inadequacy has led to the inability to ensure timely administration of primaquine for radical cure. Two main reasons could explain the inadequacy in diagnosing *P. ovale* spp. in Anhui Province. Firstly, *P. ovale* spp. is morphologically similar to *P. vivax*, leading to challenges for microscopists in accurately distinguishing between the two species, thus limiting the assessment to a qualitative analysis (Ding G et al., 2018). Secondly, the RDTs employed in Anhui Province demonstrate insufficient sensitivity in detecting *P. ovale* spp. and do not provide results for species identification (Li W et al., 2021). This shortfall is widespread among similar products on the market (Hawadak J et al., 2021). Consequently, the emergence of more sensitive point-of-care detection methods, such as Loop - Mediated Isothermal Amplification (LAMP), and Recombinase - Aided Amplification (RAA), for *P. ovale* spp. is essential for deployment. can be employed in diagnosing *P. ovale* spp. Ultimately, although the diagnosis and treatment of *P. ovale* spp. necessitate further improvement, the disease management is carried out promptly, with the "1–3–7″ indicator maintaining a satisfactory level.

Our study has three primary limitations. Firstly, being a retrospective study, it was susceptible to recall bias, which limits our ability to establish causal relationships. Additionally, the small sample size might impact the statistical conclusions. Secondly, a small number of cases, *P. ovale* spp. with follow-up periods of less than one year may lead to certain relapse events remaining unrecorded. Thirdly, the results of this study should be cautiously interpreted and not generalized to the entirety of China.

5. Conclusion

In Anhui Province, the increase in infections can be attributed to repeated exposure, which heightens the risk of contracting *P. ovale* spp. during extended stays in endemic regions, as well as to insufficient treatment of hepatic hypnozoites. In addition, healthcare facilities in the province should improve their diagnostic and treatment capacities for *P. ovale* spp., particularly by adopting more sensitive detection techniques, such as LAMP and RAA.

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Ethical approval

The current study is exempt from ethical review in accordance with Article 32 of the "Ethical Review Measures for Life Sciences and Medical Research Involving Human Subjects" issued by the Chinese government. The experiments adhered to the applicable guidelines and regulations outlined in the "National Malaria Elimination Action Plan (2010–2020)" and "Management Approach for Preventing *Re*-transmission of Malaria." Patient identifiers were anonymized before analysis.

CRediT authorship contribution statement

Tao Zhang: Writing – original draft, Validation, Resources, Methodology, Funding acquisition. **Xiaofeng Lyu:** Writing – original draft, Software, Investigation, Formal analysis, Data curation. **Xian Xu:** Validation, Project administration. **Shuqi Wang:** Resources. **Jingjing Jiang:** Resources, Investigation, Data curation. **Zijian Liu:** Investigation. **Qinshu Chu:** Software, Formal analysis. **Weidong Li:** Supervision, Conceptualization. **Duoquan Wang:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

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

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.actatropica.2025.107573.

Data availability

Data will be made available on request.

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