Epidemiological profile of Plasmodium ovale spp. imported from Africa to Anhui Province, China, 2012 – 2019

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Research

Keywords: Plasmodium ovale spp., Plasmodium ovale curtisi, Plasmodium ovale wallikeri, imported malaria, Anhui province

Posted Date: August 24th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-60169/v1

Version of Record: A version of this preprint was published on January 6th, 2021. See the published version at https://doi.org/10.1186/s12936-020-03551-8.

Abstract

Background

Although autochthonous malaria cases are no longer reported in Anhui Province, China, imported malaria has become a major health concern. The proportion of reported malaria cases caused by *Plasmodium ovale* spp. increased to levels higher than expected during 2012 to 2019, and showed two peaks, 19.69% in 2015 and 19.35% in 2018.

Methods

We conducted a case-based retrospective study, using data collected from the China Information System for Disease Control and Prevention and Information System for Parasitic Disease Control and Prevention from 2012–2019, to describe the trends and differences between *P. o. curtisi* and *P. o. wallikeri.* Epidemiological characteristics were analyzed using descriptive statistics.

Results

P. ovale curtisi and *P. ovale wallikeri* were found simultaneously circulating in 14 African countries. Among 128 patients infected with *P. ovale* spp., the proportion of mixed infection cases was 10.16%. Six cases of co-infection by *P. ovale* and *P. falciparum* were observed, each presenting with two clinical attacks (the first attack was due to *P. falciparum* and the second was due to *P. ovale spp.*) at different intervals. Accurate identification of the infecting species was achieved in only 20.00% of cases with *P. ovale* infection. At the reporting units, 32.17% and 6.96% of *P. ovale* spp. cases were misdiagnosed as *P. vivax* and *P. falciparum*, respectively.

Conclusion

Our finding indicates that the ability of *P. ovale* spp. to co-infect with other malarial species has been previously underestimated and providing rational evidence for the existence of hypnozoites in *P. ovale* spp. *P. o. curtisi* and *P. o. wallikeri* circulate simultaneously in Africa, and the incidence of *P. ovale* spp. is probably also underestimated in these source countries where the disease is endemic. Given the low rate of species identification, more sensitive point-of-care detection methods need to be developed for *P. ovale* spp., and introduced in non-endemic areas.

Background

Malaria remains a major global public health problem. In 2018, figures show that there were an estimated 228 million malaria cases, leading to 405,000 deaths worldwide [1]. Most malaria cases (93%) and deaths (94%) were in the World Health Organization (WHO) African Region, where *Plasmodium falciparum* is the most prevalent malarial parasite, accounting for an estimated 99.7% of cases [1].

In China, the National Malaria Elimination Action Plan (NMEAP) was launched in 2010, with the final objective of achieving elimination by 2020[2, 3]. Owing to the introduction of the “1-3-7 model” to deliver and monitor the elimination process [4], local transmission of malaria in Anhui, a south-eastern province of China, was successfully interrupted [5]. No autochthonous cases have been reported since 2014. By 2019, Anhui province had been malaria-free for six years and passed the sub-national malaria elimination assessment. Nevertheless, with globalization and increased international movement, the province is facing a challenge from imported malaria cases.

There were 935 imported cases of malaria reported in Anhui Province from 2012 to 2019. Interestingly, the proportion of malaria caused by *P. ovale* spp. showed two peaks, 19.69% in 2015 and 19.35% in 2018, and increased during this time to levels higher than expected. *P. ovale* is one of the four main species of parasite responsible for transmission to humans and was identified by Stevens in 1914 [6]. It has been recently confirmed by sequence analysis that *P. ovale* spp. essentially comprises two nonrecombining species, *P. ovale curtisi* and *P. ovale wallikeri* [7]. Infection due to *P. ovale* spp. has generally been considered rare and limited by geographic distribution [8]. However, the availability of more sensitive PCR diagnosis methods has revealed that its geographic distribution is larger than previously thought [7, 9]. To date, only a few epidemiological studies have investigated the respective dynamics of the *P. ovale* subspecies and there are still large gaps in our knowledge. In the present study, we conducted a case-based, retrospective, comparative study to describe the trends and differences between *P. o. curtisi* and *P. o. wallikeri.* Increased knowledge of the epidemiology of the *P. ovale* spp. will help in developing more effective public health responses.

Methods

Cases and subspecies confirmation

Patients confirmed to be infected by the parasite using a diagnostic test were defined as malaria cases (infections). In this study, whole blood samples of patients were collected prior to antimalarial treatment, and then DNA was extracted using the QIAamp DNA Mini kit (QIAGEN Inc,

Hilden, Germany) according to the manufacturer's instructions. The final diagnosis was confirmed by microscopic examination of Giemsa-stained thick and thin blood films under oil immersion at 1000× magnification, and by real-time PCR, in the Malaria Diagnostic Reference Laboratory of Anhui Province. Commercial real-time PCR kits (Shanghai BioGerm Medical Biotechnology Co., Ltd) were used, according to previously published methods [10], to discriminate between *P. o. curtisi* and *P. o. wallikeri*. Real-time PCR was performed in a 25 μL reaction volume, which contained 16 μL reaction mix, 4 μL Primers Probe OVA (c/w), and 5 μL DNA template. The reaction conditions were 95 ℃ for 5 minutes, followed by 40 cycles at 95 ℃ for 10 seconds, and 55 ℃ for 40 seconds.

Data collection

Cases of infection acquired outside the country of diagnosis were defined as "imported" [11]. As malaria is a notable disease risk in China, each imported case is mandatorily reported [12]. The basic clinical and epidemiological data of each case were collected from the China Information System for Disease Control and Prevention (CISDCP) and the Information System for Parasitic Disease Control and Prevention (ISPDCP) – a subsystem of the CISDCP, which includes age, sex, occupation, travel history, usual residence, date of onset and diagnosis, symptoms, and prognosis. At the end of the investigation, staff from the county-level Center completed an epidemiological report for disease control, the main purpose of which was to identify the origin of the infection and record other valuable information.

Data analysis

All statistical tests for data were performed using SPSS version 17.0 (SPSS Inc., Chicago, IL, USA). MapInfo 15.0 (Pitney Bowes Inc., Troy, NY, USA) compiled a thematic map of geographic distribution. The one-sample Kolmogorov-Smirnov test was used to test the normality of distribution of continuous variables. Continuous variables with normal distribution were presented as mean ± standard deviation and non-normal variables were expressed as median plus interquartile range (IQR). Means of two continuous, normally distributed variables were compared by Student's t test for independent samples. The Mann-Whitney U test was used to compare non-normal variables. Categorical data were presented by ratios and percentages. The differences in proportions were compared using Pearson χ^2 or Fisher's exact test, where appropriate. All statistical tests were considered significant with 2-sided p value < 0.05.

Results

Epidemiological profile of imported malaria in Anhui Province, 2012–2019

There were 941 cases of imported malaria reported in Anhui province from 2012 to 2019. Cross-checks of the patient identifiers revealed that 6 patients had 2 clinical episodes due to different malarial parasites after returning to China. These patients were reported twice due to different dates of onset. PCR confirmed they were mixed infections (all were *P. falciparum* co-infected with *P. ovale*). For the purpose of the study, the six cases were recorded as mixed infections, according to the date of the second clinical episode, and the number of imported malaria cases was updated to 935. *P. falciparum* was the dominant species, accounting for 733 cases (78.4%), followed by *P. ovale* (115 cases, 12.30%), *P. malariae* (38 cases, 4.06%), *P. vivax* (35 cases, 3.74%) and mixed infection (14 cases, 1.50%). Among 14 cases of mixed infection, there were 12 cases of *P. falciparum* co-infected with *P. ovale*, one of *P. falciparum* with *P. malariae*, and one of *P. malariae* with *P. ovale*. The proportion of imported cases due to *P. ovale* spp. reached a peak in 2015 (19.69%) and 2018 (19.35%), and increased from 2012 to 2018 (χ^2 = 9.626, p = 0.002, excluded 2019) (Fig. 1).

Origin of imported cases of P. ovale spp.

All 128 patients infected with *P. ovale* spp., including mixed infections, were imported from 16 countries in Africa. A total of 127 (99.22%) *P. ovale* spp. cases were successfully confirmed by PCR; one patient did not have enough blood available for PCR and was only confirmed as *P. ovale* spp. by microscopy. The top four countries of origin for these infections were Equatorial Guinea (24, 18.75%), Angola (22, 17.19%), Nigeria (18, 14.06%) and Cameroon (12, 9.38%) (Table 1). One case, infected in Angola, was found to be *P. o. curtisi* co-infected with *P. o. wallikeri*. Therefore, 129 *P. ovale* isolates were included in the analysis. Except for Ethiopia and Uganda (only one case reported), *P. o. curtisi* and *P. o. wallikeri* were simultaneously detected in all countries. The proportion of *P. o. curtisi* ranged from 39.13% to 66.67% (Fig. 2).

Table 1
Origin of imported *P. ovale* spp. in Anhui, 2012–2019.

Country	*P. ovale curtisi*		*P. ovale wallikeri*	
	N	%	N	%
Equatorial Guinea	13	19.12	11	18.33
Nigeria	11	16.18	7	11.67
Angola[a]	9	13.24	13	21.67
Cameroon	6	8.82	6	10.00
Ghana	5	7.35	3	5.00
Gabon	4	5.88	3	5.00
Zambia	3	4.41	2	3.33
Mozambique	3	4.41	2	3.33
Ivory Coast	3	4.41	4	6.67
Congo(Brazzaville)	3	4.41	2	3.33
Malawi	2	2.94	1	1.67
Guinea	2	2.94	0	0.00
Congo(Kinshasa)	2	2.94	3	5.00
Uganda	1	1.47	0	0.00
Ethiopia	1	1.47	0	0.00
Benin	0	0.00	3	5.00
Total	68	100.00	60	100.00

[a] One case, infected in Angola, did not have enough blood available to discriminate subspecies of *P. ovale* spp.

The incidence of P. ovale **spp. in the main countries of origin**

Because the number of people returning from countries of infectious origin were variable, a high proportion of *P. ovale* spp. did not necessarily reflect high incidence. In this study, we utilized the number of returnees (Anhui Statistical Yearbook, http://tjj.ah.gov.cn/ssah/qwfbjd/tjnj/index.html) from countries of infectious origin to estimate *P. ovale* spp. *and P. falciparum* incidence rates in the four main countries (Cameroon, Angola, Equatorial Guinea and Nigeria). Using this method, the estimated average annual incidence rates of *P. ovale* spp. in Cameroon, Angola, Equatorial Guinea and Nigeria were 2.48%, 0.30%, 1.87%, and 1.70%, respectively. The average annual incidence rates of *P. falciparum* in Cameroon, Angola, Equatorial Guinea and Nigeria were 9.90%, 2.86%, 8.86%, and 8.11%, respectively (Table 2).

Table 2
The incidence rates of *P. ovale* spp. and *P. falciparum* in the population returning from Africa to Anhui province

Year	*P. ovale* spp.				*P. falciparum*			
	Cameroon (%, n/N[a])	Angola (%, n/N)	Equatorial Guinea(%, n/N)	Nigeria (%, n/N)	Cameroon (%, n/N)	Angola (%, n/N)	Equatorial Guinea(%, n/N)	Nigeria (%, n/N)
2012	0.00(0/64)	0.00(0/3648)	0.50(2/397)	0.00(0/208)	3.13(2/64)	0.52(19/3648)	4.03(16/397)	2.88(6/208)
2013	2.3(1/44)	0.14(2/1427)	1.53(5/326)	3.85(3/78)	25.00(11/44)	3.22(46/1427)	10.74(35/326)	23.08(18/78)
2014	0.00(0/60)	0.51(3/593)	2.65(7/265)	2.65(4/176)	11.67(7/60)	4.72(28/593)	12.08(32/265)	7.39(13/176)
2015	2.15(2/93)	1.02(5/489)	5.38(6/112)	0.00(0/205)	1.08(1/93)	7.16(35/489)	10.71(12/112)	5.37(11/205)
2016	4.73(4/85)	1.21(3/249)	0.00(0/74)	3.41(5/147)	12.94(11/85)	16.47(41/249)	5.41(4/74)	7.48(11/147)
2017	4.76(2/42)	1.00(1/101)	3.88(2/52)	5.00(5/100)	11.90(5/42)	12.87(13/101)	11.54(6/52)	10.00(10/100)
2018	5.71(1/18)	3.69(6/163)	18.18(1/6)	0.00(0/87)	16.67(3/18)	5.52(9/163)	66.67(4/6)	13.79(12/87)
Average annual incidence (%)	2.48	0.30	1.87	1.70	9.90	2.86	8.86	8.11

[a] n was the number of reported imported cases, and N was the number of people returning from the infectious origin countries in the same year.

Epidemiological characteristics of P. o. curtisi and P. o. wallikeri

Of 128 patients infected with *P. ovale*, 113 were single-species infections, as determined by PCR confirmation. Sixty-two (62/113, 54.87%) cases were *P. o. curtisi* and the remaining fifty-one were *P. o. wallikeri* (51/113, 45.13%). Among the other 15 cases, there were 13 cases of mixed infections, 1 case with co-infection of *P. o. curtisi* and *P. o. wallikeri*, and 1 case without subspecies confirmation. The median latency period for *P. o. curtisi* (59.50 days, IQR: 23.0–192.75) was longer than *P. o. wallikeri* (34 days, IQR: 12–112.50), but the difference was not significant (P = 0.070) (Fig. 3, Table 3). There were also no significant differences between the *P. o. curtisi* and *P. o. wallikeri* groups in terms of sex, age, occupation or previous malaria (Table 3). In the present study, we detected 13 cases of *P. ovale* spp. co-infecting with other species, predominantly with *P. falciparum* (12 cases). One case involved co-infection of *P. ovale* and *P. malariae*. The rate of mixed infection was 10.16% (13/128). Of all 12 mixed *P. ovale* spp. and *P. falciparum* cases, six had only one clinical episode and six had two clinical attacks whereby the first attack was due to *P. falciparum* and the second was due to *P. ovale* spp.. The intervals between the 2 clinical episodes were 33, 56, 127, 204, 295, and 1279 days, for the six patients. Among patients with one clinical episode, 4 were infected with *P. o. wallikeri* and two were infected with *P. o. curtisi*; among patients with two clinical episodes, 3 cases were of *P. o. wallikeri* and 3 were *P. o. curtisi*.

Table 3
Epidemiologic characteristics of imported two *P. ovale* subspecies.

Variables	*P. ovale curtisi*(n = 62) N (%)	*P. ovale wallikeri*(n = 51) N (%)	P value
Sex[a]			
Male	61 (98.4)	51 (100.0)	1.000
Female	1 (1.6)	0 (0.0)	
Age(years), mean(SD)	43.84 ± 9.21	43.53 ± 7.82	0.850
Occupation[a]			
Worker	51 (82.3)	46 (90.2)	0.463
Waiter	7 (11.3)	3 (5.9)	
Other	4 (6.5)	2 (3.9)	
Previous malaria			
Yes	44 (71.0)	41 (80.4)	0.248
No	18 (29.0)	10 (19.6)	
Duration of stay overseas[a,b]			
≤ 30	0 (0.0)	1 (2.3)	0.489
≤ 180	6 (11.8)	7 (16.3)	
≤ 365	10 (19.6)	11 (25.6)	
> 365	35 (68.6)	24 (55.8)	
Latency period (days)[c], median (IQR)	59.50(23.0–192.75)	34 (12–112.50)	0.070

[a]Differences in proportions were tested by Fisher's exact test.

[b]94 cases (83.19%; 94/113) with available information of duration of stay overseas.

[c]The time elapsed, in days, between arrival in China and onset of disease was defined as the latency period. Cases showing onset of symptoms before arrival were not included in the analysis. A case with a record of 1533 days is excluded, as the authenticity is questionable.

Diagnosis of imported P. ovale **spp.**

To analyze the diagnostic data on imported *P. ovale*, *P. falciparum* was used as the control group in the study. The median interval of *P. ovale* from arrival in China to symptom onset was 49 (IQR: 16.5 − 169.5) days; from onset to the first medical visit was 1 (IQR: 0 − 3) day; and from the first medical visit to diagnosis was 1 (IQR: 0 − 3) day. The median interval of *P. falciparum* from arrival in China to symptom onset was 6 (IQR: 2 − 10) days; from onset to the first medical visit was 1 (IQR: 0 − 2) day; and from the first medical visit to diagnosis was 1 (IQR: 0–2) day. Differences in median intervals between the two species showed that *P. ovale* spp. had significantly longer interval from return to onset than *P. falciparum* ($Z = -12.947$, $p < 0.001$).

The parasite detection rates for *P. ovale* spp. and *P. falciparum* were comparable at 93.04% and 93.59%, respectively. However, the rates of species identification were significantly different between *P. ovale* spp. and *P. falciparum* ($\chi^2 = 255.841$, $p < 0.001$). Of the 115 *P. ovale* cases, only 20% of the cases (23/115) had an accurate species identification (Table 4), while 32.17% (37/115) were misdiagnosed as *P. vivax* and 6.96% (8/115) as *P. falciparum*, by the reporting units. In the remaining cases, species identification was not attempted. Among 733 *P. falciparum* cases, 86.90% of the cases (637/733) had accurate species identification, while 11.87% (87/733) could not provide the results of species identification. Only 9 cases were misdiagnosed as other malaria species or mix-infections.

Table 4
The diagnosis of imported *P. ovale* and *P. falciparum* in Anhui, 2012–2019.

Variables	*P. ovale* (n = 115) N (%)	*P. falciparum* (n = 733) N (%)	P value
Time from return to the onset (days), Median (IQR)[a]	49 (16.5, 169.5)	6(2,10)	< 0.001
Time from onset to first medical visit (days), Median (IQR)	1(0,3)	1(0,2)	0.479
Time from first medical visit to diagnosis (days), Median (IQR)	1(0,3)	1(0,2)	0.091
Parasite detection[b,c]	107(93.04)	686(93.59)	0.826
Species identification[c,d]	23 (20.00)	636 (86.77)	< 0.001

[a]Cases with onset of symptoms before arrival were not included in the analysis.

[b]The results of diagnosis came from the reporting units.

[c]Parasite detection: calculated according to whether or not a parasite was detected [13].

[d]Species identification: calculated based on the number of correctly identified species [13].

Discussion

Although *P. ovale* was identified by Stevens in 1914[6], it has received comparatively little attention in medical research. In 2010, two subspecies, *P. ovale curtisi* and *P. ovale wallikeri*, were confirmed by sequence analysis [7]. However, few studies have described their epidemiological differences, and findings have not been consistent. A recent study of imported cases to the United Kingdom showed that *P. ovale wallikeri* had a shorter latency than *P. ovale curtisi* [14], which was corroborated in a separate study of imported cases to Henan Province, China [15]. However, a third study of improted cases to Jiangsu Province, China, did not find a significant difference in latency period [16]. In the present study, although the difference is slightly short of the significance level (p = 0.070), we found evidence that the latency period of *P. ovale wallikeri* is shorter than that of *P. ovale curtisi*, but larger studies are needed to confirm this at the statistical significance threshold. In fact, the concept of a latency period in *P. ovale* spp. has been challenged based on limited experimental and clinical data supporting the hypnozoite model. Richter et al. have argued that evidence was not sufficient to unequivocally demonstrate that *P. ovale* hypnozoites are found in the human host [17]. According to another review, only 18 cases of relapse for *P. ovale* have been recorded in nearly 100 years [18]. However, a recent study provides direct evidence, using molecular methods, of the reappearance of *P. ovale curtisi* strains, in line with currently accepted relapse theory. Interestingly, the relapse of *P. ovale wallikeri* was not observed in this study [19]. In our study, there were 6 mixed cases of *P. ovale* spp. / *P. falciparum* that triggered two clinical attacks (the first attack due to *P. falciparum*, and the second due to *P. ovale* spp.). At the primary *P. falciparum* attack, patients were administered artemisinin-based drugs without primaquine, the only effective drug against dormant liver parasites [20]. The second clinical attack due to *P. ovale* spp. occurred at a different interval. The remaining *P. ovale* spp. infections were treated with primaquine after diagnosis and no reappearance of parasites in the blood was reported. Our findings provide rational evidence for the existence of hypnozoites as the origin of *P. ovale* spp. relapse events. But, based on the evidence so far, it is difficult to judge that the second episode is a relapse or late latency infection. In fact, the six mixed infection cases with two clinical episodes were the most important findings in the present study. Identifying such mixed infections is difficult in an endemic transmission setting. As China is not an endemic area for *P. ovale* spp., if the given patient does not travel abroad again, it is very easy to diagnose mixed infections. Long-term observation is also needed; among the six cases, the longest interval between two clinical episodes was 1279 days. The six cases strongly suggest that the ability of *P. ovale* spp. to co-infect with other malarial species has been underestimated because cases as such were hard to be observed in cross-sectional survey. It is worth noting that 71.0% *P. ovale curtisi* and 80.4% *P. ovale wallikeri* were associated with a previous history of malaria. It could be that more mixed infections, with two clinical attacks, may not be recorded as such, thus leading to under-reporting. So, we propose a new hypothesis that our surveillance system only captured the second clinical attack due to *P. ovale* spp. in the domestic, and did not record the first clinical episode abroad. It may lead to an increased proportion of *P. ovale* spp. Of course, we need more evidence. On the other hand, McKenzie and Bossert reported that higher overall prevalence of *P. falciparum* and *P. vivax* in a human population is associated with fewer mixed-species infections than expected [21], using point-prevalence data from 35 other studies. According to our finding, a possible explanation is that a moderate proportion of mixed infections comprising hypnozoites are undetected.

According to a study by Mehlotra et al., infection by one species of malarial parasite does not reduce susceptibility to infection by others [22]. Therefore, more mixed infections would suggest that the prevalence of *P. ovale* spp. has been underestimated. In this study, we examined the

returnees from four African countries to estimate the incidence rates of *P. ovale* and *P. falciparum*. Although it is only an estimate, taking *P. falciparum* as a reference, the data suggest that the incidence of *P. ovale* spp. was higher than expected and a growing body of evidence supports this hypothesis [23–25]. As for the geographic distribution, our findings suggest that the two subspecies, *P. o. curtisi* and *P. o. wallikeri*, have been circulating simultaneously in many Africa countries, consistent with a previous study [26].

Due to their similarities in morphology and life cycle, *P. ovale* spp. is easily and frequently misdiagnosed as *P. vivax* [27, 28]. In this study, only 20.00% of *P. ovale* cases (23/115) returned an accurate species identification; 32.17% (37/115) were misdiagnosed as *P. vivax* and 6.96% (8/115) as *P. falciparum* by the reporting units. The success rates of species identification were significantly different between *P. ovale* spp. (20.00%) and *P. falciparum* (86.77%). This may be because two rapid diagnostic tests (Pf/pan, by Wondfo and by ACCESSBIO) that are highly sensitive to *P. falciparum*, but are insensitive to *P. ovale* spp. [29, 30], have been used to provide parasite-based diagnosis in Anhui province since 2013. If *P. ovale* spp. cases were misdiagnosed as *P. vivax* or *P. falciparum*, it may have led to inappropriate case management measures and treatment regimens. Therefore, more sensitive point-of-care detection methods for *P. ovale* need to be developed and introduced in non-endemic areas.

Our study has several limitations. First, it was a case-based, retrospective study, and was subject to recall bias. Second, some differences are just outside the level of statistical significance, requiring larger studies to confirm the findings. Lastly, the data on prophylaxis use are limited, which may affect the estimated latency period of *P. ovale* spp.

Conclusion

Six *P. ovale / P. falciparum* co-infection cases were observed, which presented as two clinical attacks, indicating that the ability of *P. ovale* to co-infect with other malarial species has been previously underestimated and providing rational evidence for the existence of hypnozoites in *P. ovale* spp. *P. o. curtisi* and *P. o. wallikeri* circulate simultaneously in Africa, and the incidence of *P. ovale* spp. is probably also underestimated in these source countries where the disease is endemic.

Given the low rate of species identification, more sensitive point-of-care detection methods need to be developed for *P. ovale*, and introduced in non-endemic areas.

Declarations

Authors' contributions

SZL and WDL designed study; CY and CCT contributed to the data analysis; XFL and JJJ were responsible for data collection; SQW and JJJ participated in the sample collection and performed the laboratory studies; DQW and XX provided the administrative coordination; TZ and SQW contributed to original draft; SA and SNL provided review and editing. coordination. All authors read and approved the final version of the manuscript.

Acknowledgements

We acknowledge staff members of the municipal and county Centers for Disease Control and Prevention of Anhui Province for their contribution to data collection.

Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval

This study was approved by the Institutional Ethics Committee of Anhui Provincial Center for Disease Control and Prevention. As the study was based on a retrospective review of disease notification data, informed consent was waived. The identifiers of patients were removed before analysis.

Consent for publication

Not applicable

Funding

National Science and Technology Key Projects (2016ZX10004222-004). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript

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Figures

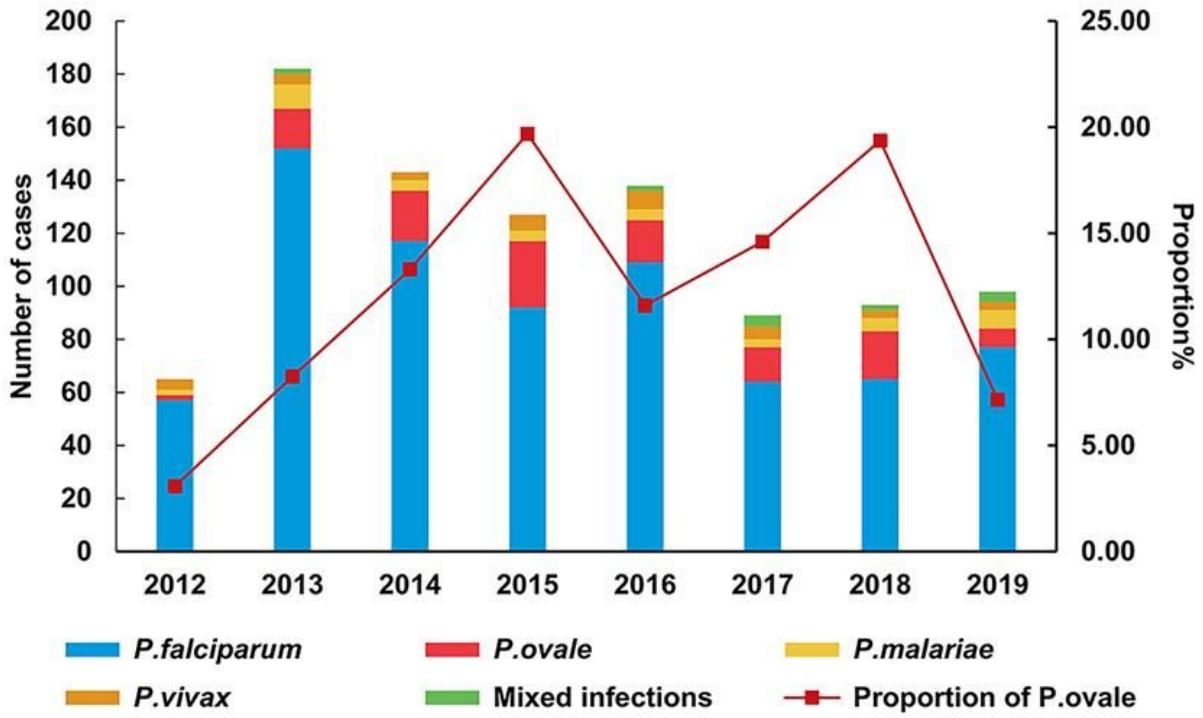

Figure 1

Imported malaria cases in Anhui Province, 2012 – 2019.

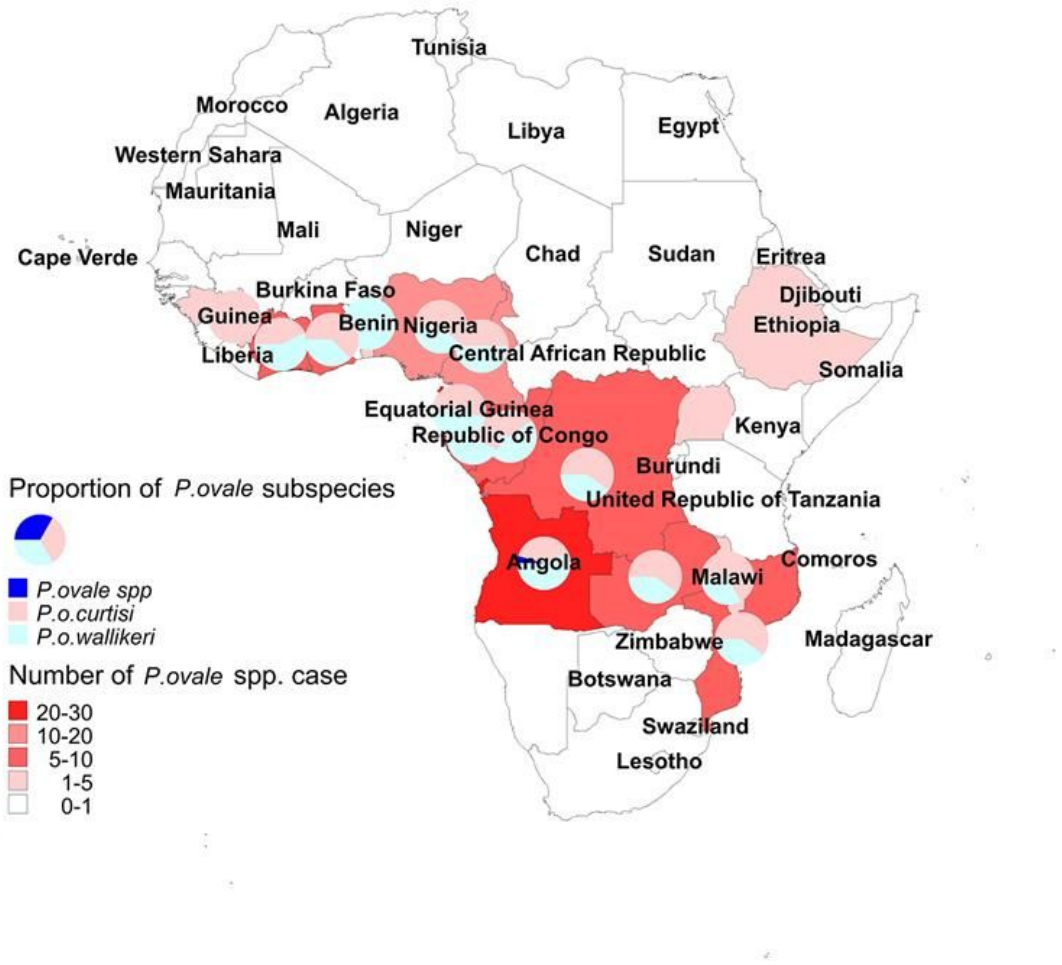

Figure 2

Geographic distribution of the origin of imported P. ovale cases in Africa, 2012 – 2019.

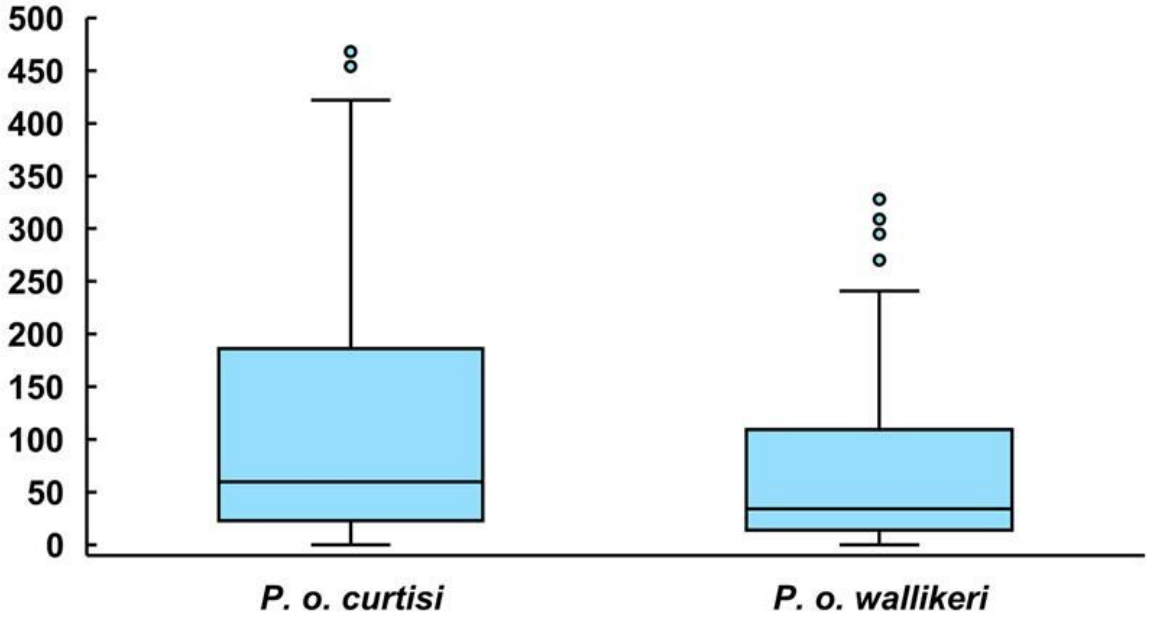

Figure 3

Latency period for P. ovale curtisi and P. ovale wallikeri cases in Anhui province, 2012-2019.