Polymorphisms analysis of the *Plasmodium ovale* tryptophan-rich antigen gene (*potra*) from imported malaria cases in Henan Province

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**Abstract**

**Background:** *Plasmodium ovale* has two different subspecies: *P. ovale curtisi* and *P. ovale wallikeri*, which may be distinguished by the gene *potra* encoding *P. ovale* tryptophan-rich antigen. The sequence and size of *potra* gene was variable between the two *P. ovale* spp., and more fragment sizes were found compared to previous studies. Further information about the diversity of *potra* genes in these two *P. ovale* spp. will be needed.

**Methods:** A total of 110 dried blood samples were collected from the clinical patients infected with *P. ovale*, who all returned from Africa in Henan Province in 2011–2016. The fragments of *potra* were amplified by nested PCR. The sizes and species of *potra* gene were analysed after sequencing, and the difference between the isolates were analysed with the alignment of the amino acid sequences. The phylogenetic tree was constructed by neighbour-joining to determine the genetic relationship among all the isolates. The distribution of the isolates was analysed based on the origin country.

**Results:** Totally 67 samples infected with *P. o. wallikeri*, which included 8 genotypes of *potra*, while 43 samples infected with *P. o. curtisi* including 3 genotypes of *potra*. Combination with the previous studies, *P. o. wallikeri* had six sizes, 227, 245, 263, 281, 299 and 335 bp, and *P. o. curtisi* had four sizes, 299, 317, 335 and 353 bp, the fragment sizes of 299 and 335 bp were the overlaps between the two species. Six amino acid as one unit was firstly used to analyse the amino acid sequence of *potra*. Amino acid sequence alignment revealed that *potra* of *P. o. wallikeri* differed in two amino acid units, MANPIN and AITPIN, while *potra* of *P. o. curtisi* differed in amino acid units TINPIN and TITPIS. Combination with the previous studies, there were ten subtypes of *potra* exiting for *P. o. wallikeri* and four subtypes for *P. o. curtisi*. The phylogenetic tree showed that 11 isolates were divided into two clusters, *P. o. wallikeri* which was then divided into five sub-clusters, and *P. o. curtisi* which also formed two sub-clusters with their respective reference sequences. The genetic relationship of the *P. ovale* spp. mainly based on the number of the dominant amino acid repeats, the number of MANPIN, AITPIN, TINPIN or TITPIS. The genotype of the 245 bp size for *P. o. wallikeri* and that of the 299 and 317 bp size for *P. o. curtisi* were commonly exiting in Africa.

**Conclusion:** This study further proved that more fragment sizes were found, *P. o. wallikeri* had six sizes, *P. o. curtisi* had four sizes. There were ten subtypes of *potra* exiting for *P. o. wallikeri* and four subtypes for *P. o. curtisi*. The genetic
polymorphisms of potra provided complementary information for the gene tracing of P. ovale spp. in the malaria elimination era.

**Keywords:** Plasmodium ovale curtisi, Plasmodium ovale wallikeri, Plasmodium ovale tryptophan-rich antigen (potra), Amino acid unit, Subtype

**Background**

*Plasmodium ovale* was first described in 1922 [1], as the fourth malaria parasite of humans [2, 3]. Generally, a *P. ovale* infection is of low parasitaemia, and the morphology of the parasite is similar to *Plasmodium vivax*. Also, it frequently presents as a mixed infection with the other *Plasmodium* species [4–9]. As a result, *P. ovale* attracted less attention compared to other species, and its prevalence has apparently been underestimated. It has long been considered predominantly found in Africa and some islands of Western Pacific [10, 11], with confirmed cases occasionally found in other endemic regions [12, 13].

Currently, *P. ovale* spp. may be classified into two different subspecies by molecular genotyping: *P. o. curtisi* (classic type) and *P. o. wallikeri* (variant type) [14]. The nuclear genome sequences further confirmed that the two species were genetically different, but morphologically indistinct [15], and their duration of latency were seemingly different [16]. Both species were considered to exist sympatrically in Africa and Asia, and even both parasites were infected simultaneously [17–21].

Because of the generally low parasitaemia of *P. ovale* infections, sensitive molecular methods to detect and identify the two subspecies must be used in future investigations, with polymorphic markers as a method to discriminate the different strains. Many protocols showed that the SS rRNA genes [17, 22, 23] were suitable for identification but not for genotyping. The recent study showed that the gene encoding *P. ovale* tryptophan-rich antigen (potra) could be used to distinguish the two *P. ovale* subspecies [14]. The sequence and size of the tryptophan-rich antigen gene was variable among the *P. ovale* subspecies (potra and powtra) [14]. A nested PCR detection assay was exploited to discriminate the species by the size of the amplified fragments (299 or 317 bp for potra; 245 bp for powtra), where the conserved sequences were chosen as primers for these two genes [19]. Additionally, a semi-nested PCR protocol was developed by Tanomsing et al. [24] with which the two *P. ovale* subspecies could be discriminated efficiently, and more fragment sizes were found comparing with previous studies, the 299 bp fragment was overlapping between the two subspecies. This would invalidate amplified fragment size difference, as a means of distinguishing between *P. o. curtisi* and *P. o. wallikeri*. The amplified fragment size variations resulted from differences in the number of repeated units, which suggested that a broader range of size variants might occur. In this study, more variations of potra gene were observed.

**Methods**

**Sample collection and DNA extraction**

Dried blood spots on filter paper (Whatman 3M) were collected from patients returned from Africa with *P. ovale* infection before treatment. All the patients were diagnosed by nested PCR and microscopy. All the dried blood spots were labelled with a unique identification number, air-dried and individually placed in plastic bags with desiccant and stored at −20 °C until laboratory analysis. DNA was extracted from the dried blood spots using a QIAamp DNA mini kit (Qiagen, Germany).

**Nested PCR amplification and DNA sequencing**

The fragments of potra was amplified with nested PCR using the primers as described previously [14, 19]. The amplified products were identified by agarose gel electrophoresis. Bidirectional sequencing was performed for the secondary potra PCR products using the secondary primers by Sangon Biotech Co Ltd (Shanghai, China).

**Sequencing alignments and analysis**

All the genes sequences were analysed with multiple sequence alignment using the Clustal X software. HM594180–HM594183 [19], KF018430–KF018433 [24] and KX417700–KX417704 [25] from the GenBank would be as the reference sequences of *P. ovale* spp. Phylogenetic trees were constructed using the Molecular Evolutionary Genetics Analysis (MEGA) 6.06.

**Results**

**Amplification of the potra gene of Plasmodium ovale spp.**

A total of 110 dried blood samples, from patients returned from Africa to Henan Province with *P. ovale* infection, were collected. The amplified nested PCR products of the potra gene of 110 samples were blasted in the GenBank. The blast data showed that 67 samples infected with *P. o. wallikeri* and 43 samples infected with *P. o. curtisi*. More fragment sizes of the potra gene from this study were found comparing with the previous reports. *P. o. wallikeri* had five different sizes including 227, 245, 263, 281 and 299 bp, while *P. o. curtisi* had three
sizes including 299, 317 and 335 bp. Also, the amplified fragment size differed as a result of differences in 18 bases of units, with the overlap of 299 bp between the two species (Fig. 1). The size of 245 bp was the main type for \( P. o. \) wallikeri, accounting for 82.1% (55/67), and the 299 bp was the main size for \( P. o. \) curtisi, accounting for 67.4% (29/43). The number of isolates for each size was shown in the Table 1.

Oguike reported that the sizes of the \( \text{potra} \) gene was 245 bp for \( P. o. \) wallikeri and 299 bp and 317 bp for \( P. o. \) curtisi [19] in Congo, Uganda and Equatorial Guinea. While there were three sizes (245, 299 and 335 bp) for \( P. o. \) wallikeri and three sizes (299, 317 and 353 bp) for \( P. o. \) curtisi in the study of Tanomsing et al. [24]. Combination with the previous studies, \( P. o. \) wallikeri had six sizes, 227, 245, 263, 281, 299 and 335 bp, and \( P. o. \) curtisi had four sizes, 299, 317, 335 and 353 bp, the fragment sizes of 299 and 335 bp were the overlaps between the two species (shown in Table 1).

Genotypes of \( \text{potra} \) of \( \text{Plasmodium ovale} \) spp.
There were 8 genotypes of the \( \text{potra} \) gene for the 67 isolates infected with \( P. o. \) wallikeri and three genotypes of \( \text{potra} \) for the 43 isolates infected with \( P. o. \) curtisi. The sizes of 277, 245 and 263 bp for \( P. o. \) wallikeri all had two different subtypes. The sequences of the 11 genotypes of \( \text{potra} \) gene were deposited in GenBank under accession number MG588144-MG588154. For \( P. o. \) wallikeri, the genotype of MG588146 was the same with that of the reference sequences HM594180 and HM594181, but the reference sequences KF018430 and KF018431 were different with any of MG588144-MG588151. For \( P. o. \) curtisi, the genotype of MG588152 was the same with that of the reference sequences HM594182 and KF018433, and the genotype of MG588153 was the same with that of the reference sequence HM594183. Combination with the previous studies, there were ten genotypes of \( \text{potra} \) exited for \( P. o. \) wallikeri, and four genotypes for \( P. o. \) curtisi. The number of isolates for each genotype was shown in the Table 2.

Alignment of the translated amino acid sequence of \( \text{potra} \) fragments
Interestingly, the translated amino acid sequence of \( \text{potra} \) fragments were composed with multiple amino acid units, and six amino acids was considered as a unit. For \( P. o. \) wallikeri, it was mainly composed with nine kinds of amino acid units, TANPIN, MANPIN, MAKPIN, TITPIN, AITPIN, AITPIK, TITSIN, TITPMN and TITPIS. While \( P. o. \) curtisi had six kinds of amino acid units, TANPIN, AANPIN, TINPIN, KINPIN, TITPIN and TITPIS. The two subspecies of \( P. ovale \) had three common amino acid units, TANPIN, TITPIN and TITPIS. Amino acid sequence alignment revealed that the types of \( P. o. \) wallikeri differed in two amino acid units, MANPIN and AITPIN, while the types of \( P. o. \) curtisi differed in amino acid units TINPIN and TITPIS. The amplified fragment size differed as a result of differences in amino acid of repeat units. The dominant amino acid repeats of \( \text{potra} \) were showed in Table 2. As the same with the genotypes of \( \text{potra} \), there were 10 subtypes exiting for \( P. o. \) wallikeri and 4 subtypes for \( P. o. \) curtisi. The detail information is shown in Fig. 2 and Table 2.

Phylogenetic relationship among \( \text{potra} \) subtype families
Neighbour-joining was used to cluster the \( \text{potra} \) gene sequences. The 11 genotypes were classified into two clusters, eight genotypes infected with \( P. o. \) wallikeri were

Table 1 Main findings of studies that sequenced the sizes of \( \text{potra} \) fragments

| Authors        | \( \text{Plasmodium ovale curtisi} \) | \( \text{Plasmodium ovale wallikeri} \) |
|---------------|--------------------------------------|-------------------------------------|
| Oguike et al. [19] | 299 bp (no data)                      | 245 bp (no data)                    |
| Tanomsing et al. [24] | 299 bp (n = 3)                       | 245 bp (n = 12)                     |
|                | 317 bp (n = 1)                       | 299 bp (n = 6)                     |
|                | 353 bp (n = 1)                       | 335 bp (n = 2)                     |
| Zhou et al. * | 299 bp (n = 29)                      | 227 bp (n = 3)                     |
|                | 317 bp (n = 12)                      | 245 bp (n = 55)                    |
|                | 335 bp (n = 2)                       | 263 bp (n = 4)                     |
|                |                                      | 281 bp (n = 4)                     |
|                |                                      | 299 bp (n = 1)                     |

* This study

Fig. 1 Identification of the fragment of \( \text{potra} \) gene by agarose gel electrophoresis. Lanes 1–5: the fragment sizes of 227, 245, 263, 281 and 299 bp for \( P. ovale \) wallikeri, respectively; lanes 6–8: the fragment sizes of 299, 317 and 335 bp for \( P. ovale \) curtisi, respectively. M molecular marker

![alignment of amino acid sequence](image-url)
as one cluster and other three genotypes infected with *P. o. curtisi* formed the other one. Meanwhile the cluster of *P. o. wallikeri* was classified into five sub-clusters, distinctive phylogenetic relationship presented between the eight genotypes of *P. o. wallikeri*. The sequence 1-227, formed a sub-cluster with reference sequences HM594180, HM594181 and KX417700, having the same 2 repeats of AITPIN. The sequences 2-227 and 3-245 existed in the same sub-cluster with a closer genetic relationship, having the same repeat of MANPIN. The sequences 4-245 and 5-263 formed a sub-cluster with reference sequence KX417704, having the same two repeats of MANPIN. The sequences 6-263 and 7-281 formed another sub-cluster with reference sequences KF018430 and KF018431, having the same three repeats of AITPIN. Having significantly difference sequences, the three genotypes of *P. o. curtisi* also formed two sub-clusters. The sequence 9-299 formed a sub-cluster with reference sequences, having the same repeat of TITPIS. The sequences 10-317 and 11-335 had a closer genetic relationship, which formed another sub-cluster with reference sequences, having the same two repeats of TITPIS (Fig. 3).

**Distribution of the *Plasmodium ovale* spp.**
All the 110 *P. ovale* patients returned from twenty countries of Africa, and 70.0% (77/110) were from Angola, Congo, Equatorial Guinea, Liberia and Nigeria. Only one patient originated from Algeria, Tanzania, Sudan, Libya and Ivory Coast. 82.1% (55/67) *P. o. wallikeri* infection were the same subtype with 245 bp size of the *potra* gene, and this type was identified in 16 countries except Gabon, Algeria, Tanzania and Libya; three isolates (4.5%, 3/67) with 227 bp size originated from Congo and Liberia; four isolates (6.0%, 4/67) of 263 bp size were from Angola, Liberia and Gabon; 4 isolates

### Table 2 Analysis of the distinct *potra* fragments amplified from 11 genotypes of *P. ovale* and the reference sequences

| Sample | *Plasmodium ovale* spp. | Size (bp) | Dominant amino acid repeats | No. of samples | GenBank accession no. |
|--------|------------------------|-----------|-----------------------------|----------------|----------------------|
| 1-227  | *wallikeri*            | 227       | (MANPIN)0(AITPIN)2           | 1              | MG588144             |
| 2-227  | *wallikeri*            | 227       | (MANPIN)1(AITPIN)1           | 2              | MG588145             |
| 3-245  | *wallikeri*            | 245       | (MANPIN)1(AITPIN)2           | 1              | MG588146             |
| 4-245  | *wallikeri*            | 245       | (MANPIN)2(AITPIN)1           | 54             | MG588147             |
| 5-263  | *wallikeri*            | 263       | (MANPIN)2(AITPIN)2           | 3              | MG588148             |
| 6-263  | *wallikeri*            | 263       | (MANPIN)1(AITPIN)3           | 1              | MG588149             |
| 7-281  | *wallikeri*            | 281       | (MANPIN)2(AITPIN)3           | 4              | MG588150             |
| 8-299  | *wallikeri*            | 299       | (MANPIN)2(AITPIN)4           | 1              | MG588151             |
| HM594180a | *wallikeri*     | 245       | (MANPIN)1(AITPIN)2           | –              | –                    |
| HM594181a | *wallikeri*     | 245       | (MANPIN)1(AITPIN)2           | –              | –                    |
| KFO18430a | *wallikeri*     | 299       | (MANPIN)3(AITPIN)3           | –              | –                    |
| KFO18431a | *wallikeri*     | 335       | (MANPIN)5(AITPIN)3           | –              | –                    |
| 9-299  | *curtisi*              | 299       | (TINPIN)3(TITPIS)1           | 29             | MG588152             |
| 10-317 | *curtisi*              | 317       | (TINPIN)3(TITPIS)2           | 12             | MG588153             |
| 11-335 | *curtisi*              | 335       | (TINPIN)4(TITPIS)2           | 2              | MG588154             |
| HM594182a | *curtisi*      | 299       | (TINPIN)3(TITPIS)1           | –              | –                    |
| HM594183a | *curtisi*      | 317       | (TINPIN)3(TITPIS)2           | –              | –                    |
| KFO18432a | *curtisi*      | 353       | (TINPIN)6(TITPIS)1           | –              | –                    |
| KFO18433a | *curtisi*      | 299       | (TINPIN)3(TITPIS)1           | –              | –                    |

* GenBank accession no.; –: no date

### Fig. 2 Amino acid sequence alignment of the distinct *potra* fragments amplified from 11 genotypes of *Plasmodium ovale* and the reference sequences.

*a* *P. o. wallikeri*; *b* *P. o. curtisi*. Six amino acids were one amino acid unit which were underlined. *P. o. wallikeri* was mainly composed with nine kinds of amino acid units, TANPIN, MANPIN, MAKPIN, TITPIN, AITPIN, AITPIK, TITSIN, TITPMN and TITPIS. While *P. o. curtisi* had six kinds of amino acid units, TANPIN, AANPIN, TINPIN, KINPIN, TITPIN and TITPIS. Boxed sequences represented the dominant amino acid repeats that differed in the number of amino acid units. The sequences of *P. o. wallikeri* were characterized by two amino acid units, MANPIN and AITPIN. The sequences of *P. o. curtisi* were characterized by amino acid units TINPIN and TITPIS.
(6.0%, 4/67) of 281 bp size were from Congo, Liberia, Nigeria and Sierra Leone; only one isolate of 299 bp size was from Congo. In contrast, the predominant type of *P. o. curtisi* (67.4%, 29/43) was the *potra* gene with 299 bp size, which originated from 12 countries; 27.9% (12/43) with 317 bp size and 4.7% (2/43) with 335 bp size were also identified from nine countries and two countries, respectively (Table 3).

**Discussion**

Malaria elimination is a long-term goal to be achieved worldwide. As one species of human Plasmodium, the identification of *P. ovale* is more widespread than formerly known. *Plasmodium ovale*, like *P. vivax*, has hypnozoites that cause relapses [26, 27], and it consists of two different subspecies: *P. ovale curtisi* and *P. ovale wallikeri* [14]. Therefore, the differentiation of the two...
P. ovale species, especially with respect of molecular phylogeny will need to be better understood.

In 2011, Oguike et al. [19] published the discrimination of the two P. ovale subspecies by the size of the amplified fragments of the potra gene (299 or 317 bp for P. o. curtisi; 245 bp for P. o. wallikeri), using nested PCR. Although this technique was specific for P. ovale spp., the sizes of the amplified fragment varied with the number of repeat units, which reduced the discrimination between species: for P. o. curtisi, 299, 317 and 335 bp, and for P. o. wallikeri, 245, 299 and 335 bp [24]. Tanomsing et al. [24] suggested the number of potra size variations might be more than those evaluated and this speculation was confirmed in this study. Using the same primes and method, more fragment sizes were identified in this study, while some sizes overlapped between the two subspecies: for P. o. curtisi having sizes 299, 317 and 335 bp, and for P. o. wallikeri having sizes 227, 245, 263, 281 and 299 bp. The results of the three studies [19, 24] were also combined, as shown in Table 1. Four different sizes for P. o. curtisi and six sizes for P. o. wallikeri have been reported, and that the fragment sizes of 299 and 335 bp were overlaps between the two species. As more samples were analysed, it was likely that the number of potra size variants would be more than expected. Conceivably, more size variants may be identified in future studies.

Potra gene was used to discriminate the two P. ovale species, because the tryptophan-rich antigen was encoded by a repeat pattern of variable length 3-amino acid [14]. Sutherland et al. [14] and Oguike et al. [19] had also proposed that the potra gene of P. o. curtisi (pocitra) could be identified by the pattern of the six amino acids of the repeat region, TITPIS, while the potra gene of P. o. wallikeri (powtra) were different in two non-synonymous positions. By alignment of the amino acid sequence of potra fragments, 11 genotypes of potra were found from the 110 isolates in this study. Combination with the previous studies of Oguike et al. [19] and Tanomsing et al. [24], showed ten subtypes of potra gene for P. o. wallikeri and four subtypes for P. o. curtisi, and 14 genotypes of potra gene were under analysis. This study showed that the amino acid sequence of potra fragments were composed with multiple amino acid units, six amino acids were as one unit. There was different dominant amino acid repeat of potra for the two P. ovale species, which could be used to discriminate the subtype of P. ovale

| Origin country | Plasmodium ovale wallikeri | | | | | | | | Total |
|----------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
|                | 227 bp     | 245 bp     | 263 bp     | 281 bp     | 299 bp     | Subtotal | 299 bp     | 317 bp     | 335 bp     | Subtotal |
| Angola         | 0          | 12         | 2          | 0          | 0          | 14       | 5          | 2          | 0          | 7         | 21         |
| Congo          | 2          | 7          | 0          | 1          | 11         | 11       | 2          | 1          | 1          | 4         | 15         |
| Equatorial Guinea | 0          | 8          | 0          | 0          | 0          | 8        | 5          | 1          | 0          | 6         | 14         |
| Liberia        | 1          | 7          | 1          | 1          | 0          | 10       | 4          | 1          | 0          | 5         | 15         |
| Nigeria        | 0          | 4          | 0          | 1          | 0          | 5        | 4          | 3          | 0          | 7         | 12         |
| Ghana          | 0          | 1          | 0          | 0          | 0          | 1        | 3          | 1          | 0          | 4         | 5          |
| Cameroon       | 0          | 3          | 0          | 0          | 0          | 3        | 0          | 0          | 1          | 1         | 4          |
| The Republic of Guinea | 0          | 2          | 0          | 0          | 0          | 2        | 0          | 1          | 0          | 1         | 3          |
| Uganda         | 0          | 3          | 0          | 0          | 0          | 3        | 0          | 0          | 0          | 3         | 3          |
| Sierra Leone   | 0          | 1          | 0          | 1          | 0          | 2        | 1          | 0          | 0          | 1         | 3          |
| Benin          | 0          | 2          | 0          | 0          | 0          | 2        | 0          | 0          | 0          | 2         | 2          |
| Chad           | 0          | 1          | 0          | 0          | 0          | 1        | 0          | 1          | 0          | 1         | 2          |
| Mozambique     | 0          | 1          | 0          | 0          | 0          | 1        | 1          | 0          | 0          | 1         | 2          |
| Zambia         | 0          | 1          | 0          | 0          | 0          | 1        | 1          | 0          | 0          | 1         | 2          |
| Gabon          | 0          | 0          | 1          | 0          | 0          | 1        | 1          | 0          | 0          | 1         | 2          |
| Algeria        | 0          | 0          | 0          | 0          | 0          | 0        | 0          | 1          | 0          | 1         | 1          |
| Tanzania       | 0          | 0          | 0          | 0          | 0          | 0        | 1          | 0          | 0          | 1         | 1          |
| Sudan          | 0          | 1          | 0          | 0          | 0          | 1        | 0          | 0          | 0          | 0         | 1          |
| Libya          | 0          | 0          | 0          | 0          | 0          | 0        | 1          | 0          | 0          | 1         | 1          |
| Ivory Coas     | 0          | 1          | 0          | 0          | 0          | 1        | 0          | 0          | 0          | 0         | 1          |
| Total          | 3          | 55         | 4          | 4          | 1          | 67       | 29         | 12         | 2          | 43        | 110        |
spp. The repeat of six amino acids as one unit to analyse the difference of the genotypes between the two P. ovale species was first reported, which could make the results more clear and simple. The sizes of 277, 245 and 263 bp for P. o. wallikeri all had two different subtypes, and this phenomenon did not find in P. o. curtisi.

The sizes of the reference sequences KX417700–KX417704 [25] from Genbank were short for discriminating the differences of dominant amino acid repeats. In our study, the genetic relationship of the P. ovale spp. was analysed by the neighbour-joining tree mainly based on the number of the dominant amino acid repeats including MANPIN, AITPIN, TINPIN or TITPIS.

The distribution of the two P. ovale spp. was different. The isolates of P. o. wallikeri was more than that of P. o. curtisi, and the genotype of the 245 bp size was the predominant type for P. o. wallikeri in most Africa countries, but the other genotypes were less. For P. o. curtisi, the genotypes of the 299 and 317 bp size were commonly in Africa and the genotype of 335 bp size was less.

Molecular epidemiological studies on genetic diversity of Plasmodium vivax have been based mainly on single copy polymorphic genes which code for parasite surface antigens such as circumsporozoite protein (msp), merozoite surface protein-1 (msp-1) and merozoite surface protein 3 alpha (msp 3α) [28]. Pvcsp comprises of central domain of tandem repeated sequences flanked by two non-repeated conserved sequences [29–32]. Two types of repeat elements, either VK210 or VK247 types were detected in clinical isolates of P. vivax and thus pvcsp serves as a useful tool for genotyping [33, 34]. Potra has the similar characteristics with pvcsp, which also could be used for parasite genotyping.

**Conclusions**

Considering the change of malaria epidemiology and the approaching of malaria elimination, P. ovale spp. deserves more attention. Molecular techniques are a good tool for detecting and identifying the two P. ovale subspecies and their relative distribution.

**Abbreviations**

Potra: Plasmodium ovale tryptophan-rich antigen; Sp: plural form of species; PCR: polymerase chain reaction; NCBI: national center for biotechnology information; DNA: deoxyribonucleic acid; MEGA: molecular evolutionary genetics analysis.

**Authors' contributions**

RMZ was responsible for the molecular genetic analysis and data interpretation and drafted the manuscript. YL participated in sample detection and data analysis. HWZ and BLX conceived the study and revised the manuscript. FH revised the manuscript. SUL participated in sample collection and sample detection. YLZ, YD and DLL provided the administrative coordination. CYY and FH revised the manuscript. All authors read and approved the final manuscript.

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**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 first/secondary author on reasonable request.

**Ethics approval and consent to participate**

The purpose of this study was demonstrated to participants before sampling, who agreed to participate in the survey, and the consent was obtained from them. Approval of the study was obtained from the Ethical Committee of Henan Province Center for Disease Control and Prevention, prior to commencement of the study.

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