IJMPES International Journal of
Medical Parasitology & Epidemiology Sciences

http://ijmpes.com
doi 10.34172/ijmpes.2021.18
Vol. 2, No. 3, 2021, 60-64
eISSN 2766-6492

Original Article

*Pf*LDH Detected in Etawa Crossbred Goats Using Polymerase Chain Reaction Methods

Didik Sumanto[1*], Sayono Sayono[1], Martini Martini[2], Wahyu Handoyo[1,3], Tri Dewi Kristini[1,4]

[1]Department of Epidemiology, Faculty of Public Health, Universitas Muhammadiyah Semarang, Indonesia
[2]Faculty of Public Health, Universitas Diponegoro, Semarang, Indonesia
[3]Public Health Center of Semarang, Central Java Province, Indonesia
[4]Health Office of Central Java Province, Indonesia

Abstract

Introduction: Livestock is the potential source of blood feed for Anopheles mosquitoes. Infectious Anopheles bites enter *h-Plasmodium* sporozoites in the body of livestock. In the erythrocytic phase, the parasite produces several enzymes, one of which is parasite lactate dehydrogenase (pLDH). The parasite needs this enzyme to help the glycolysis process of pyruvate into lactic acid.

Methods: In this cross-sectional study, the presence of pLDH in blood specimens of Etawa crossbreed goats was explored. Samples of the stored biological material in the form of dried blood in Whatman™ 1001 125 filter paper included 97 specimens. *Pf*LDH was detected using the PCR method.

Results: One sample previously reported to contain *P. falciparum* trophozoites under microscopic examination showed positive results in *Pf*LDH testing.

Conclusion: The parasite of *P*. falciparum was found capable of living up to the erythrocytic phase and forming *Pf*LDH in the Etawa crossbreeds.

Keywords: *Pf*LDH, Etawa crossbred goats, Malaria, PCR-methods

Received: April 11, 2021, **Accepted**: September 13, 2021, **ePublished**: October 1, 2021

Introduction

Domestic goats have the potential to be sources of blood for zoophilic *Anopheles* mosquitoes. The bite of a malaria vector introduces human-Plasmodium (*h-Plasmodium)* sporozoites in the target animal (1,2). When the parasite's life in the host reaches the erythrocytic phase, several metabolic products are produced, including histidine-rich protein (HRP), parasite lactate dehydrogenase (pLDH), and aldolase (3–5). All these three are markers of the presence of *h-Plasmodium* in the host's body. Although it is not the gold standard for malaria testing (6), the appearance of these markers still provides important information regarding the transmission that occurs.

The pLDH enzyme is produced by the *h-Plasmodium* parasite when it enters the erythrocytic phase (7). The enzyme formation is required to support the life of the parasite itself through the process of glycolysis which converts pyruvate to lactic acid (8). The pLDH emerges along with the process of *h-Plasmodium* infection that occurs in the host. The pLDH enzyme in human cases lasts relatively long in the blood, and it may even be detected several days after the patient receives treatment (9). Information on *h-Plasmodium* life in domestic animals has not been widely disclosed.

Transmission of *h-Plasmodium* to vertebrate hosts cannot be separated from the role of the *Anopheles* spp mosquito as a malaria vector (10-12). Some vectors are zoophilic, meaning that they prefer animal blood to human's (13). It is the zoophilic nature to search for blood feed sources in animals. The presence of a group of livestock in one cage can provide more inviting environment for zoophilic *Anopheles* (14). The search for blood feed sources among female mosquitoes is of a vital necessity to sustain life (15). Inevitable foraging activities expose the domestic animals to the attacks by female zoophilic *Anopheles* (13,16).

Many studies have failed to reveal the presence of *h-Plasmodium* in animals and livestock. Identifying *h-Plasmodium* in a group of domestic cattle in Sumba and Fakfak, however, has confirmed the fact that this parasite can live in the livestock (17). A similar finding in goats was reported in Jatirejo by microscopic examination of thick blood smears (18), although some practitioners still doubt it. The presence of *h-Plasmodium* in domestic

*Corresponding Author: Didik Sumanto, Email: didik.24272@gmail.com

livestock is a new warning signal for those living in, especially, malaria-endemic and pre-elimination areas since it indicates that the parasite is also capable of breeding in non-human vertebrate hosts.

Malaria pre-elimination areas must strive to maintain zero local cases for three years to achieve malaria elimination status (19,20). Many factors must be taken care of for the elimination program to be fruitful. Promotive, preventive, and curative efforts must be undertaken separately and/or in synergy (21). Patient management alone is not sufficient to control the spread of malaria (22). It requires best efforts to control the vector of transmission and suppress the potential for wider transmission (23,24).

If *h-Plasmodium* can live in the livestock, it means that the malaria control program is not enough to manage the sufferers as well as their vectors and, therefore, the program must also include the management of livestock rearing. As the result, detecting the presence and survival strategies of *h-Plasmodium* in domestic livestock assumes an added importance. This study, therefore, aimed to detect the presence of pLDH in stored biological materials that previously had been reported to contain the *h-Plasmodium* parasite.

Materials and Methods

Materials

The subject of the study was biological a material stored in a dried blood spot in Whatman™ 1001 125 filter paper. The previously reported samples contained *h-Plasmodium* Human Plasmodium-like was reported previously as trophozoite form on thick blood smears(25). This biological material, having been stored in the Public Health Laboratory of the Universitas Muhammadiyah Semarang, was the residue from previous research. As for the information about the specimens, the blood samples were obtained from the Etawa crossbreed goats aged at least three months and kept in Jatirejo village. The total number of the biological materials was 97.

Study Design

An observational study was conducted to explore the emergence of *p*LDH in blood of goats from the malaria pre-elimination areas by performing laboratory testing.

Polymerase Chain Reactions Testing

DNA Isolation

Spots of dried blood on filter paper were cut with a sterile perforator and put into a 1.5 mL microtube containing 200 µL of InstaGene Matrix (Bio-Rad Laboratories, USA). Then it was incubated at 56°C for 30 minutes. The sample was vortexed for 10 seconds at 15 and 30 minutes during incubation. The specimen was heated at 100°C for 8 minutes, and centrifuged at 15 000 g for 2 minutes. The supernatant was taken carefully and stored at minus 20°C as a result of DNA isolation.

DNA Amplification

Primer (Macrogen Humanizing Genomics) was diluted according to the directions in reagent kit. The base sequences for *Plasmodium falciparum* were 5'ATGGCACCAAAAGCAAAA3' (Forward) and 5'TTAAGCTAATGCCTTCATTCTC3' (Reverse), while those for *Plasmodium vivax* were 5'CCATGGCTATGACGCCGAAACCCAAAATTG3' (Forward) and 5'CTCGAGAATGAGCGCCTTCATCC3' (Reverse) (26). DNA template (2.5 µL), forward–reverse primer (1 µL each), 2x Taq plus Tiangen KT205 master-mix (5 µL), and nuclease-free water (3 µL) were added to the content of PCR tube. Amplification was done in Bio-Rad Thermal Cycler-100. Pre-denaturation at 94°C for 3 minutes, denaturation at 94°C for 30 seconds, annealing at 53.5°C for 30 seconds, extension at 68°C for 1 minute, final extension at 68°C for 5 minutes, and cooling at 4°C with 40 cycles of repetition were all performed.

Electrophoresis

Agarose weighing 1 g was added to 100 mL of 1% TBE solution, mixed well, and heated in the oven for 1-2 minutes. While the mixture was hot, 25 µL of GelRed nucleic acid stain (Biotium Inc, USA) was added to it and homogenized. Agarose solution was poured into a mold with a well of some samples tested. The comb was removed from the agarose after it has cooled. The agarose gel was placed on Consort-Electrophoresis and filled with 1% TBE solution. Ten microliter markers of 100 base pairs were inserted into the first well from the left side, followed by negative control and PCR test samples. Electrophoresis was processed at 100 V for 60 minutes. It was difficult to get positive controls; therefore, only the negative control was used. The negative control was a parasite-free sample on microscopic examination.

Result Reading

Agarose gel was installed in the Mini Illuminator. The target DNA appeared in orange colour under UV. The photo documentation was taken from the electrophoresis results by a mini illuminator. The concentration of the sample was calculated on agarose gel pictures by GelAnalyzer 19.1 application. The pLDH weights were compared with a pLDH reference between 800–1000 base pairs by using an appropriate marker (27).

Results

Most of the goat blood samples contained no *h-Plasmodium* and only four samples were found positive (Table 1). The parasites were detected to be trophozoites of *P. vivax* and *P. falciparum* (Figure 1) (18). The *P. falciparum* samples determined positive by previous microscopic test were also found positive *Pf*LDH by PCR method (Figure 2).

Table 1. Test Results of pLDH Compared to Results From Previous Microscopic Test

Microscopically (18)	**Number**	***Pv*LDH**	***PfLDH***
Findings			
Positive			
P. vivax	3	0	0
P. falciparum	1	0	1
Negative	93	0	0
Total	97	0	1

Discussion

The study site had long been known as a malaria-endemic area (28,29). The last reported local case occurred in October 2018 (30). Great efforts had been made by the government, including treatment of the confirmed malaria-positive patients and execution of a vector control program by performing an insecticide residual spraying (IRS) for the patient's house and surrounding areas (31). The distribution of long-lasting insecticide-treated nets (LLINs) has also been implemented (32). The main program of the Purworejo District Health Office to control malaria is the 'Migration Surveillance' (33). All programs were very successful in reducing malaria cases in Purworejo to zero until early 2021 (34). In June 2021, however, a significant increase in malaria cases was detected in Bener sub-district (35).

The efforts for controlling malaria have been comprehensive, targeting the patient and the vector of transmission. However, there is one more neglected factor, that is, the foraging behaviour of female *Anopheles* vectors in Purworejo. The previous vector survey examined three species including *Anopheles maculatus, Anopheles subpictus*, and *Anopheles aconitus* in Jatirejo village (36). These species are zoo-anthropophilic – in other words, they like animal blood as well as human blood (13,37,38). The bite prevention strategies to ensure human health included the execution of IRS program (31) and distribution of LLINs (32); however, no similar strategies were developed to protect animals against Anopheles bites. The zoophilic Anopheles gives malaria parasites opportunities to transfer from mosquitoes to animals.

The discovery of *Pf*LDH in the blood of Etawa crossbreed goats is an important piece of information. The presence of the pLDH enzyme in the blood indicates a biological activity of the parasite's life that converts pyruvate into lactic acid. The pLDH enzyme can be produced by all five species of *h-Plasmodium* (39–41). Detection of pLDH has a sensitivity of up to 60.1% (42), and even a sensitivity of 94% has been reported (43). The specificity of pLDH has been found to be better than that of the HRP2 enzyme (44). The emerging of pLDH was easily detected in blood samples with parasitemia levels of 0.2-10% (45,46) and 75 parasites/μL (47,48).

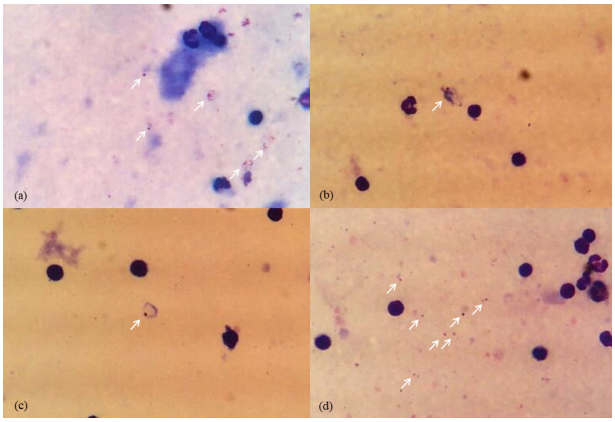

Figure 1. Three Samples Contained the Late Phase of *P. vivax* Trophozoites in Rough Rings (a) and Irregular (b, c) With Rounded Nuclei, One Sample Found the Early Trophozoites of *P. falciparum* in Dots, Commas, and Exclamation Points (d) (18).

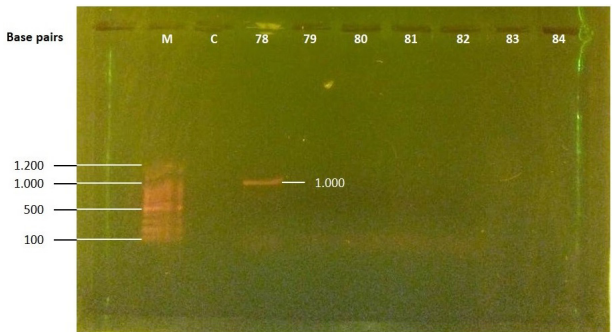

Figure 2. Electrophoresis Results of *Pf*LDH Testing on Agarose Gel. An orange color spot of a sample (78) which is parallel to the marker color spot (M) with a size of 1000 base pairs is seen, while the other samples are negative.

Several assumptions made about the presence of pLDH in the blood of Etawa crossbreed goat have been found valid, suggesting that *h-Plasmodium* already inhibited its body. The pLDH enzyme is formed on the first day after erythrocytes is invaded by merozoites from the liver (7). Then the level of enzyme increases continuously until it reaches a peak at 24-48 hours in the erythrocytic phase (49,50) and, as a result, the enzyme becomes detectable in the blood. The process of pLDH formation in the human host continues until the beginning of gametocyte stage and its presence can still be detectable several days after treatment (9) or, put it another way, as long as there are living parasites in the blood. The results from studies on livestock groups have not been widely disclosed, but the above-mentioned findings may apply to human case.

The local case had not been detected in the study site in the previous three years, but it was recommended that everyone should remain vigilant. The detection of *Pf*LDH in goat blood indicated that live parasites had already inhibited the animal before the present study was conducted. This implied that people of Jatirejo village were still under the threat of malaria disease. Therefore, it was recommended that human contact with vectors at night should be strictly minimized. It was found that executing

IRS programs in the focus areas as well as improving the efficacy of the distributed LLINs mosquito nets were likely the best solutions to deal with the problem. It was also suggested that the public awareness about the disease should be constantly promoted in order to help people adopt healthy behaviors and avoid the risks of malaria transmission.

Conclusion

In sum, the discovery of *Pf*LDH in Etawa crossbreeds was a piece of interesting, valuable information that needed to be further analysed. The presence of *Pf*LDH in the blood of Etawa Crossbreed goat was suggestive of the presence of infectious zoophilic vectors at the study site. Therefore, it was highly recommended that a continuing effort should be undertaken in order to monitor the biological activity of the human *Plasmodium* in domestic animals.

Acknowledgments

The authors would like to thank all those working in the Public Health Laboratory, Faculty of Public Health, Universitas Muhammadiyah Semarang for facilitating the implementation of laboratory testing.

Authors' Contribution

DS: conception, data collecting, writing manuscript; SY: study design, methods, data analysis; MM: methods, writing manuscript; WH: laboratory analysis, data interpretation; and TDK: data collecting, data interpretation.

Conflict of Interests

The authors declare that they have no conflict of interests.

Ethical Issues

Ethical clearance number 029/EC/FK/2020 was issued by the Commission of Ethics Health-Research Faculty of Medicine, Universitas Muhammadiyah Semarang

References

1. CDC. Live cycle of *Plasmodium* spp. 2017. Available from: http://www.dpd.cdc.gov/dpdx.
2. Miller K. Plasmodium Life Cycle. Available from: http://www.intellectualventureslab.com/invent/plasmodium-life-cycle.
3. Pal-Bhowmick I, Andersen J, Srinivasan P, Narum DL, Bosch J, Miller LH. Binding of aldolase and glyceraldehyde-3-phosphate dehydrogenase to the cytoplasmic tails of *Plasmodium falciparum* merozoite duffy binding-like and reticulocyte homology ligands. mBio. 2012;3(5):e00292-12. doi:10.1128/mBio.00292-12
4. Poti KE, Sullivan DJ, Dondorp AM, Woodrow CJ. HRP2: transforming malaria diagnosis, but with caveats. Trends Parasitol. 2020;36(2):112-126. doi:10.1016/j.pt.2019.12.004
5. Atchade PS, Doderer-Lang C, Chabi N, et al. Is a *Plasmodium* lactate dehydrogenase (pLDH) enzyme-linked immunosorbent (ELISA)-based assay a valid tool for detecting risky malaria blood donations in Africa? Malar J. 2013;12:279. doi:10.1186/1475-2875-12-279
6. U.S. Department of Health & Human Service. Malaria Diagnosis. cdc.gov. 2015. Available from: https://www.cdc.gov/malaria/diagnosis_treatment/diagnosis.htmL.
7. Salinas ND, Tolia NH. Red cell receptors as access points for malaria infection. Curr Opin Hematol. 2016;23(3):215-223. doi:10.1097/moh.0000000000000219
8. Penna-Coutinho J, Cortopassi WA, Oliveira AA, França TC, Krettli AU. Antimalarial activity of potential inhibitors of *Plasmodium falciparum* lactate dehydrogenase enzyme selected by docking studies. PLoS One. 2011;6(7):e21237. doi:10.1371/journal.pone.0021237
9. Tjitra E, Suprianto S, McBroom J, Currie BJ, Anstey NM. Persistent ICT malaria P.f/P.v panmalarial and HRP2 antigen reactivity after treatment of *Plasmodium falciparum* malaria is associated with gametocytemia and results in false-positive diagnoses of *Plasmodium vivax* in convalescence. J Clin Microbiol. 2001;39(3):1025-1031. doi:10.1128/jcm.39.3.1025-1031.2001
10. Lestari EW, Sukowati S, Soekidjo S. Vektor Malaria di Daerah Bukit Menoreh Purworejo Jawa Tengah. Pusat Penelitian dan Pengembangan Ekologi Kesehatan. Jakarta; 2008. Available from: http://repository.litbang.kemkes.go.id/1275/. Accessed December 22, 2021.
11. Barodji TBD, Boesri H, Sudini, Sumardi. Bionomik Vektor dan Situasi Malaria di Kecamatan Kokap, Kabupaten Kulonprogo, Yogyakarta. J Ekol Kesehat. 2003;2(2):209-216. Available from: http://ejournal.litbang.kemkes.go.id/index.php/jek/article/view/1393.
12. Ipa M, Prasetyowati H, Yuliasih Y. Konfirmasi Anopheles Barbirostris Sebagai Vektor Malaria Di Waikabubak Melalui Deteksi Protein Circum Sporozoite. J Vector Borne Dis Stud. 2012;4(1):1-6.
13. Arifianto RP, Masruroh D, Habib MJ, et al. Identifikasi dan Analisis Bionomik Vektor Malaria Anopheles sp. di Desa Bangsring Kecamatan Wongsorejo, Banyuwangi. Acta Vet Indones. 2018;6(1):44-50. doi:10.29244/avi.6.1.44-50
14. Mulyono A, Alfiah S, Sulistyorini E, Negari KS. Hubungan Keberadaan Ternak dan Lokasi Pemeliharaan Ternak terhadap Kasus Malaria di Provinsi NTT (Analisis Lanjut Data Riskesdas 2007). J Vektora. 2013;5(2):73-77.
15. Dambach P, Schleicher M, Korir P, et al. Nightly biting cycles of *Anopheles* species in rural Northwestern Burkina Faso. J Med Entomol. 2018;55(4):1027-1034. doi:10.1093/jme/tjy043
16. Gunathilaka N, Denipitiya T, Hapugoda M, Abeyewickreme W, Wickremasinghe R. Determination of the foraging behaviour and blood meal source of malaria vector mosquitoes in Trincomalee district of Sri Lanka using a multiplex real time polymerase chain reaction assay. Malar J. 2016;15:242. doi:10.1186/s12936-016-1279-5
17. Munirah M, Wahyuni S, Wahid I, Hamid F. The discovery of human *Plasmodium* among domestic animals in West Sumba and Fakfak, Indonesia. F1000Res. 2021;10:645. doi:10.12688/f1000research.53946.1
18. Sumanto D, Hadisaputro S, Adi MS, Susanti S, Sayono S. Parasit *Plasmodium* sp pada Ternak Kambing Etawa di Daerah Endemik Malaria Kabupaten Purworejo. J Ekol Kesehat. 2021;20(1):36-44. doi:10.22435/jek.v20i1.4092
19. Subuh M. Panduan Pemeliharaan Eliminasi Malaria. Jakarta: Kementerian Kesehatan Republik Indonesia; 2017:1-36.
20. Kusriastuti R. Buku Saku Menuju Eliminasi Malaria. Jakarta: Kementerian Kesehatan Republik Indonesia; 2011:1-30.
21. Rahayu A, Santoso B, Sasmita HI, Sadar M. Metode Pengendalian Terpadu Nyamuk Vektor Penyakit Filariasis dan Malaria Dengan Teknik Serangga Mandul (TSM). 2017. Available from: http://repo-nkm.batan.go.id/2220/.
22. Sihotang V. Buku Saku Talaksana Kasus Malaria. Jakarta: Kementerian Kesehatan Republik Indonesia; 2017:1-38. Available from: https://persi.or.id/wp-content/uploads/2020/11/bukusaku_malaria.pdf.
23. Setiyaningsih R, Alfiah S, Garjito TW, Heriyanto B. Assesment penyakit tular vektor malaria di Kabupaten Banyumas. Media

Litbangkes. 2015;25(2):1-6.
24. Muhadir A. Pedoman Pengendalian Vektor Malaria. Jakarta: Kementerian Kesehatan Republik Indonesia; 2014:1-50.
25. Sumanto D, Hadisaputro S, Adi MS, Susanti S. Human-*Plasmodium* like in domestic-goat blood in malaria endemic areas in Purworejo Indonesia. J Commun Dis. 2021;53(4):148-52. doi:10.24321/0019.5138.202185
26. Jain P, Chakma B, Singh NK, Patra S, Goswami P. Aromatic surfactant as aggregating agent for aptamer-gold nanoparticle-based detection of *Plasmodium* lactate dehydrogenase. Mol Biotechnol. 2016;58(7):497-508. doi:10.1007/s12033-016-9946-x
27. Shin HI, Kim JY, Lee WJ, et al. Polymorphism of the parasite lactate dehydrogenase gene from *Plasmodium vivax* Korean isolates. Malar J. 2013;12:166. doi:10.1186/1475-2875-12-166
28. Barcus MJ, Laihad F, Sururi M, et al. Epidemic malaria in the Menoreh hills of Central Java. Am J Trop Med Hyg. 2002;66(3):287-292. doi:10.4269/ajtmh.2002.66.287
29. Setyorini A. Kejadian Malaria di Kaligesing Tahun 2016. Purworejo: Puskesmas Kaligesing; 2016:7-10.
30. Bayu B. Data Kejadian Malaria Kecamatan Kaligesing Tahun 2018. Purworejo; 2018:10-15.
31. Setiyaningsih R, Trapsilowati W, Mujiyono M, Lasmiati L. Malaria Vector Control in Endemic Area, Purworejo Regency, Indonesia. Balaba J Litbang Pengendali Penyakit Bersumber Binatang Banjarnegara [Internet]. 2018;14(1):1-12. Available from: http://ejournal2.litbang.kemkes.go.id/index.php/blb/article/view/290
32. Sihotang RV. Panduan Pelaksanaan Pekan Kelambu Anti Nyamuk Massal di Daerah Fokus. Jakarta: Kementerian Kesehatan Republik Indonesia; 2017:1-44. Available from: https://ptvz.kemkes.go.id/produk/malaria.
33. Ikawati B, Isnani T, Wijayanti T, Wahyudi BF, Raharjo J, Sunaryo S, Sholichah Z. Peran lintas program, lintas sektor, dan masyarakat dalam eliminasi malaria di kawasan bukit menoreh. Vektora: Jurnal Vektor dan Reservoir Penyakit. 2020;12(2):119-32.
34. Prabowo Y. Buku Saku Kesehatan Tahun 2021 Triwulan 1. https://dinkesjatengprov.go.id/. Semarang: Dinas Kesehatan Provinsi Jawa Tengah; 2021:43. Available from : https://dinkesjatengprov.go.id/v2018/storage/2021/05/Buku_Saku_Kes_tw1_2021_Final.pdf.
35. Prabowo Y. Buku Saku Kesehatan Tahun 2021 Triwulan 2. https://dinkesjatengprov.go.id/. Semarang: Dinas Kesehatan Provinsi Jawa Tengah; 2021:43. Available from: https://dinkesjatengprov.go.id/v2018/storage/2021/08/1_Buku_Saku_Kes_tw2_2021_Final-1.pdf.
36. Putranto NT, Handoyo W, Sumanto D. Keragaman dan Kepadatan Vektor Anopheles sp di Jatirejo Purworejo. J Kesehat Masy Indones. 2020;15(2):39-41. doi:10.26714/jkmi.15.2.2020.39-41
37. Marcombe S, Maithaviphet S, Bobichon J, et al. New insights into malaria vector bionomics in Lao PDR: a nationwide entomology survey. Malar J. 2020;19(1):396. doi:10.1186/s12936-020-03453-9
38. Tananchai C, Pattanakul M, Nararak J, Sinou V, Manguin S, Chareonviriyaphap T. Diversity and biting patterns of *Anopheles* species in a malaria endemic area, Umphang Valley, Tak province, western Thailand. Acta Trop. 2019;190:183-192. doi:10.1016/j.actatropica.2018.11.009
39. Baker J, McCarthy J, Gatton M, et al. Genetic diversity of *Plasmodium falciparum* histidine-rich protein 2 (PfHRP2) and its effect on the performance of PfHRP2-based rapid diagnostic tests. J Infect Dis. 2005;192(5):870-877. doi:10.1086/432010
40. Brown WM, Yowell CA, Hoard A, et al. Comparative structural analysis and kinetic properties of lactate dehydrogenases from the four species of human malarial parasites. Biochemistry. 2004;43(20):6219-6229. doi:10.1021/bi049892w
41. McCutchan TF, Piper RC, Makler MT. Use of malaria rapid diagnostic test to identify *Plasmodium knowlesi* infection. Emerg Infect Dis. 2008;14(11):1750-1752. doi:10.3201/eid1411.080480
42. Kyabayinze DJ, Zongo I, Cunningham J, et al. HRP2 and pLDH-based rapid diagnostic tests, expert microscopy, and PCR for detection of malaria infection during pregnancy and at delivery in areas of varied transmission: a prospective cohort study in Burkina Faso and Uganda. PLoS One. 2016;11(7):e0156954. doi:10.1371/journal.pone.0156954
43. Atchade PS, Doderer-Lang C, Chabi N, et al. Is a *Plasmodium* lactate dehydrogenase (pLDH) enzyme-linked immunosorbent (ELISA)-based assay a valid tool for detecting risky malaria blood donations in Africa? Malar J. 2013;12:279. doi:10.1186/1475-2875-12-279
44. Maltha J, Guiraud I, Lompo P, et al. Accuracy of PfHRP2 versus Pf-pLDH antigen detection by malaria rapid diagnostic tests in hospitalized children in a seasonal hyperendemic malaria transmission area in Burkina Faso. Malar J. 2014;13:20. doi:10.1186/1475-2875-13-20
45. Makler MT, Ries JM, Williams JA, et al. Parasite lactate dehydrogenase as an assay for *Plasmodium falciparum* drug sensitivity. Am J Trop Med Hyg. 1993;48(6):739-741. doi:10.4269/ajtmh.1993.48.739
46. Oduola AM, Omitowoju GO, Sowunmi A, et al. *Plasmodium falciparum*: evaluation of lactate dehydrogenase in monitoring therapeutic responses to standard antimalarial drugs in Nigeria. Exp Parasitol. 1997;87(3):283-289. doi:10.1006/expr.1997.4251
47. Verma P, Biswas S, Mohan T, Ali S, Rao DN. Detection of histidine rich protein & lactate dehydrogenase of *Plasmodium falciparum* in malaria patients by sandwich ELISA using in-house reagents. Indian J Med Res. 2013;138(6):977-987.
48. Garedaghi Y. Parasitic protozoan among restaurant workers in Tabriz (East Azerbaijan province) Iran. Res J Biol Sci. 2011;6(6):272-4.
49. Basco LK, Marquet F, Makler MM, Le Bras J. *Plasmodium falciparum* and *Plasmodium vivax*: lactate dehydrogenase activity and its application for in vitro drug susceptibility assay. Exp Parasitol. 1995;80(2):260-271. doi:10.1006/expr.1995.1032
50. Biomed Penyelidikan Antarabangsa. Potensi Biomarker Dan Aplikasi Mereka Untuk Pengesanan Rapid Dan Boleh Dipercayai Malaria. forensicsciencetechniciandegree.com. 2022;1-15. Available from: https://may.forensicsciencetechniciandegree.com/potential-biomarkers-227658.