ORIGINAL ARTICLE

# Molecular Prevalence of *Entamoeba histolytica* and *Entamoeba dispar* Infection among Aboriginal Communities in the Southern Region of Perak, Malaysia

**Saidin S[a]*, Adibah AB[a], Poh KB[a], Khairuddin R[a], Md-Zain BM[b]**

[a]*Dept. of Biology, Faculty of Science and Mathematics, Universiti Pendidikan Sultan Idris, Perak, Malaysia.*

[b]*Dept. of Biological Sciences and Biotechnology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, Selangor, Malaysia*

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**Corresponding Author**
Syazwan Saidin,
Department of Biology, Faculty of Science and Mathematics, Universiti Pendidikan Sultan Idris, 35900 Tanjong Malim, Perak, Malaysia
Tel No: +6015-48797743
Email : syazwan@fsmt.upsi.edu.my

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## ABSTRACT

**INTRODUCTION**: In Malaysia, there are little up-to-date data on the prevalence of *Entamoeba* spp. and most of the previous studies were carried out using the conventional insensitive method of stool microscopic examinations. **MATERIALS AND METHODS**: A cross-sectional study was conducted from September 2018 to Mac 2019 in the three villages of Orang Asli communities at Slim River, Perak using the molecular assay. Risk factors associated with *Entamoeba* spp. infection was assessed by analysing questionnaire responses obtained from the study participants. **RESULTS**: From the 55 stool samples collected, molecular prevalence revealed 7.3% and 9.1% *E. histolytica* and *E. dispar* infection respectively. Infection was due to indiscriminate defecation and not washing hands after playing with soil or gardening, as well as associated with gastrointestinal symptoms. **CONCLUSION**: This study showed that there is still an ongoing transmission of *Entamoeba* spp. among the studied population, thus warrants the implementation of specific prevention and control strategies of this infection in Malaysia.

## INTRODUCTION

Over 0.1 million deaths were reported annually due to amoebiasis caused by an anaerobic parasitic protozoan known as *Entamoeba histolytica*.[1] In Southeast Asia, this disease is mostly found among the low socio-economic rural area communities with poor hygiene practices.[2,3] One of the affected countries is Malaysia which showed a prevalence of *Entamoeba* spp complex infection in different states that ranges from 0.4% to 83.9% using conventional microscopy technique.[4,5] However, this conventional diagnostic technique using microscopy was found to be insensitive to discriminate between *Entamoeba* species. Accurate diagnosis through the determination of true prevalence is very crucial for better management and prevention of amoebiasis, especially among vulnerable groups. Therefore, the objective of this study is to determine the prevalence of pathogenic *E. histolytica* along with both non-pathogenic *E. dispar* and *E. moshkovskii* infections using a molecular approach among Orang Asli communities in the southern Perak region as well as to identify potential associated risk factors.

## MATERIALS AND METHODS

A cross-sectional study was conducted from September 2018 to Mac 2019 on the molecular prevalence of *Entamoeba* spp. infections among participants from the Southern region of Perak with or without symptoms of amoebiasis. The study location was randomly chosen from the available district list provided by the Department of Orang Asli Development (JAKOA) taking into consideration the villages had to be located in a rural area, have more than 100 residents, and have transport accessibility. The sample size was calculated based on Kish et al.[6] considering the prevalence at 3.2%[3] and 95% level of confidence with absolute precision ($d$)= 0.05.

Approximately a thumb-size fresh stool sample was collected from each participant. About 0.2 g of the stool sample was used for genomic DNA extraction of *Entamoeba* using the QIAamp Fast DNA Stool Mini Extraction Kit (Qiagen, Germany). Single round multiplex PCR targeting small subunit ribosomal RNA gene was used for genetic characterization of *E. histolytica*, *E. dispar,* and *E. moshkovskii* according to protocols provided by Hamzah et al.[7] To confirm the species descriptions obtained by PCR, all of the targeted size amplicons were sequenced using respective primer pair based on methodology from Hamzah et al.[7] and subjected to homology search using BLAST database. GenBank accession numbers X56991, Z49256, and AF149906 for *E. histolytica*, *E. dispar,* and *E. moshkovskii* respectively, were used as reference sequences in the analyses.

For the final analysis, only stool samples with complete questionnaire data were included. For descriptive analysis, the percentage rate was used to determine the prevalence of *E. histolytica*, *E. dispar,* and *E. moshkovskii* as well as the characteristics of the studied population. *Entamoeba* spp. infection was assigned as the dependent variable while demographic (age, gender, ethnic and tribe, etc.), socioeconomic (occupational, marital status, family household size and income, education level, etc.), environmental (water supply, toilet facilities, animal contact, personal hygiene habits, etc.) as well as clinical symptoms (at the time of sample collection such as diarrhoea, vomiting, abdominal pain, blood in stool, etc.) were assigned as independent variables. A Pearson's chi-square test ($X^2$) was used to test the associations between *Entamoeba* spp. infections with the explanatory independent variables. Univariate analysis was used to determine the potential associations between *E. histolytica*, *E. dispar,* and *E. moshkovskii* infections with the independent variables. The odds ratios (OR) and 95% confidence intervals were computed with the level of statistical significance were set as *p-value* < 0.05.

## RESULTS

Throughout this study, a total of 55 eligible participants aged between 2 and 74 years old were recruited. Among these stool samples, 7.3% (4/55) were positive for *E. histolytica* and 9.1% (5/55) were positive for *E. dispar*. No evidence of the presence of *E. moshkovskii* among the participants.

Table 1 shows a univariate analysis of demographics and the prevalence of *Entamoeba* spp. infection. Based on the result, we found that the socio-demographic factors were independently connected to the infection. Likewise, no significant differences were observed in the overall prevalence of *Entamoeba* spp. infection between age groups. A total of 4 participants were confirmed to be infected with *E. histolytica*. Two of them were less than 15 years old. A total of 5 participants were infected with *E. dispar* where only 1 participant was less than 15 years old. There was no association between being non-educated and *E. histolytica* infection (OR=1.1, 95% CI=0.190, 6.351; $p$=0.916).

With regards to the environmental factors (Table 2), we observed participants who did not wash hands after gardening or playing with soil (OR=3.922; 95% CI=0.718, 21.42; $p$<0.001) more than three times more prone to acquire *E. histolytica* as compared to those who practiced proper personal hygiene. Participants who defecated indiscriminately in the river or bush were 2.5 times more likely to be infected with *E. histolytica* (OR=2.588; 95% CI=0.468, 14.302; $p$=0.011). Having improper sewage disposal outdoor did not increase the risk of *E. histolytica* infection (OR=0.49; 95% CI=0.371, 0.649; $p$=0.049). Additionally, none of the environmental factors assessed were associated with *E. dispar* infection.

Among the gastroenteritis symptoms, participants who had experienced vomiting were most probably being infected with *E. dispar* (OR=1.225; 95% CI=0.789, 1.092; $p$=0.049). However, *E. histolytica* infection did not show any association with diarrhoea or other gastroenteritis symptoms such as nausea, abdominal pain, watery stool, and blood or mucus stool.

## DISCUSSION

Conventional microscopy techniques are commonly used in prevalence studies of amoebiasis in Malaysia but are inadequate for *E. histolytica* detection.[8,9] This technique is

**Table 2:** Univariate analysis of selected environmental factors and subject's clinical symptoms associated with *Entamoeba histolytica* and *Entamoeba dispar* infection among participants

| Variables | *Entamoeaba histolytica* | | | | *Entamoeba dispar* | | | |
|---|---|---|---|---|---|---|---|---|
| | **No. of examined** | **% infected** | **OR (95%, CI)** | ***P*-value** | **No. of examined** | **% infected** | **OR (95%, CI)** | **p-value** |
| **Drinking untreated water** | | | | | | | | |
| Yes | 45 | 8.9 | 0.804 (0.702, 0.921) | 0.328 | 45 | 8.9 | 1 | 0.912 |
| No | 10 | 0.0 | 1 | | 10 | 10.0 | 1.025 (0.649, 1.619) | |
| **Bathing and washing in the river** | | | | | | | | |
| Yes | 30 | 10.0 | 1.882 (0.336, 10.535) | 0.394 | 30 | 10.0 | 1.150 (0.377, 3.505) | 0.797 |
| No | 25 | 4.0 | 1 | | 25 | 8.0 | 1 | |
| **Not washing hands after playing with soil or gardening** | | | | | | | | |
| Yes | 4 | 75.0 | 3.922 (0.718, 21.42) | <0.001* | 4 | 25.0 | 1.175 (0.754, 1.831) | 0.250 |
| No | 51 | 2.0 | 1 | | 51 | 7.8 | 1 | |
| **Close contact with domestic animals** | | | | | | | | |
| Yes | 43 | 4.7 | 1 | 0.156 | 43 | 9.3 | 1.100 (0.177, 6.851) | 0.918 |
| No | 12 | 16.7 | 1.608 (0.598, 4.324) | | 12 | 8.3 | 1 | |
| **Indiscriminate defecation** | | | | | | | | |
| Yes | 21 | 19.0 | 2.588 (0.468, 14.302) | 0.011* | 21 | 9.5 | 1.033 (0.489, 2.183) | 0.930 |
| No | 34 | 0.0 | 1 | | 34 | 8.8 | 1 | |
| **Sewage disposal** | | | | | | | | |
| Outdoor | 29 | 13.8 | 0.49 (0.371, 0.649) | 0.049* | 29 | 13.8 | 2.500 (0.424, 14.748) | 0.200 |
| Common drainage | 26 | 0.0 | 1 | | 26 | 3.8 | 1 | |
| **Eating with hand** | | | | | | | | |
| Yes | 55 | 7.3 | [a] | [a] | 55 | 9.1 | [a] | [a] |
| No | 0 | 0.0 | | | 0 | 0.0 | | |
| **Consuming raw vegetables** | | | | | | | | |
| Yes | 54 | 7.4 | 0.980 (0.943, 1.019) | 0.777 | 54 | 9.3 | 0.980 (0.942, 1.020) | 0.750 |
| No | 1 | 0.0 | 1 | | 1 | 0.0 | 1 | |
| **Eating fresh fruits** | | | | | | | | |
| Yes | 55 | 7.3 | [a] | [a] | 55 | 9.1 | [a] | [a] |
| No | 0 | 0.0 | | | 0 | 0.0 | | |
| **Diarrhoea** | | | | | | | | |
| Yes | 24 | 8.3 | 1.137 (0.415, 3.118) | 0.790 | 24 | 8.3 | 1 | 0.863 |
| No | 31 | 6.5 | 1 | | 31 | 9.7 | 1.100 (0.360, 3.365) | |
| **Vomiting** | | | | | | | | |
| Yes | 2 | 0.0 | 1 | 0.687 | 2 | 50.0 | 1.225 (0.789, 1.902) | 0.040* |
| No | 53 | 7.5 | 0.961 (0.909, 1.016) | | 53 | 7.5 | 1 | |
| **Nausea** | | | | | | | | |
| Yes | 3 | 0.0 | 1 | 0.618 | 3 | 33.3 | 1.200 (0.771, 1.867) | 0.133 |
| No | 52 | 7.7 | 0.941 (0.879, 1.008) | | 52 | 7.7 | 1 | |
| **Abdominal pain** | | | | | | | | |
| Yes | 18 | 16.7 | 2.824 (0.512, 15.558) | 0.061 | 18 | 5.6 | 1 | |
| No | 37 | 2.7 | 1 | | 37 | 10.8 | 1.700 (0.282, 10.234) | 0.525 |
| **Watery stool** | | | | | | | | |
| Yes | 17 | 5.9 | 1 | 0.791 | 17 | 11.8 | 1.167 (0.558, 2.441) | 0.645 |
| No | 38 | 7.9 | 1.255 (0.219, 7.187) | | 38 | 7.9 | 1 | |
| **Blood or mucus stool** | | | | | | | | |
| Yes | 13 | 7.7 | 1.020 (0.568, 1.832) | 0.947 | 13 | 7.7 | 1 | |
| No | 42 | 7.1 | 1 | | 42 | 9.5 | 1.200 (0.194, 7.414) | 0.841 |

CI, Confident interval; OR, Odd ratio; Reference group marked as OR=1

[a]Cornfield 95% CI for odds ratio is not accurate due to the low number

**Table 1:** Socio-demographic features of the study participants and their association with *Entamoeba histolytica* and *Entamoeba dispar* infection among the participants

| Variables | *Entamoeaba. histolytica* | | | | *Entamoeaba. dispar* | | | |
|---|---|---|---|---|---|---|---|---|
| | No. of examined | % infected | OR (95%, CI) | *P*-value | No. of examined | % infected | OR (95%, CI) | p-value |
| **Age group** | | | | | | | | |
| <15 | 16 | 12.5 | 1.451 (0.537, 3.922) | 0.339 | 17 | 5.9 | 1 | 0.580 |
| >15 | 39 | 5.1 | 1 | | 38 | 10.5 | 1.600 (0.265, 9.670) | |
| **Gender** | | | | | | | | |
| Male | 21 | 4.8 | 1 | 0.573 | 21 | 9.5 | 1.033 (0.489, 2.183) | 0.930 |
| Female | 34 | 8.8 | 1.569 (0.278, 8.861) | | 34 | 8.8 | 1 | |
| **Marital status** | | | | | | | | |
| Single | 19 | 10.5 | 1.333 (0.491, 3.261) | 0.500 | 19 | 5.3 | 1 | 0.473 |
| Married | 36 | 5.6 | 1 | | 36 | 11.1 | 1.800 (0.300, 10.798) | |
| **Education** | | | | | | | | |
| Educated | 15 | 6.7 | 1 | 0.916 | 40 | 10 | 1.400 (0.229, 8.542) | 0.702 |
| Non-educated | 40 | 7.5 | 1.098 (0.190, 6.351) | | 15 | 6.7 | 1 | |
| **Occupation** | | | | | | | | |
| Working | 22 | 9.1 | 1.216 (0.445, 3.319) | 0.672 | 22 | 18.2 | 3.200 (0.548, 18.699) | 0.056 |
| Non-working | 33 | 6.1 | 1 | | 33 | 3.0 | 1 | |
| **Household monthly income** | | | | | | | | |
| <RM500 | 50 | 8.0 | 0.902 (0.824, 0.987) | 0.511 | 50 | 8.0 | 1 | 0.373 |
| >RM500 | 5 | 0.0 | 1 | | 5 | 20 | 1.150 (0.736, 1.796) | |
| **Household members** | | | | | | | | |
| <8 | 48 | 6.3 | 1 | 0.444 | 48 | 10.4 | 0.860 (0.769, 0.962) | 0.370 |
| >8 | 7 | 14.3 | 1.176 (0.662, 2.090) | | 7 | 0.0 | 1 | |
| **Type of water supply** | | | | | | | | |
| Safe | 43 | 9.3 | 0.765 (0.657, 0.890) | 0.273 | 43 | 9.3 | 1.100 (0.177, 6.851) | 0.918 |
| Unsafe | 12 | 0.0 | 1 | | 12 | 8.3 | 1 | |
| **The presence of toilet at household** | | | | | | | | |
| Yes | 27 | 11.1 | 2.118 (0.380, 11.790) | 0.282 | 27 | 3.7 | 1 | 0.172 |
| No | 28 | 3.6 | 1 | | 28 | 14.3 | 2.600 (0.441, 15.312) | |

CI, Confident interval; OR, Odd ratio; Reference group marked as OR=1

time-consuming and needs experts to be able to differentiate between pathogenic and non-pathogenic *Entamoeba* species. Therefore, the current study adopted a more rapid approach using the molecular method to examine *Entamoeba* spp. infection among the Orang Asli community in the selected study location.

Our results showed the overall prevalence rate of *Entamoeba* spp. infection was 16.4%; with *E. dispar* infection (9.1%) was higher than *E. histolytica* (7.3%). This finding was comparable to previous local studies on *Entamoeba* infection among Orang Asli communities, which reported a prevalence rate between 17.6% - 22.5%.[3] For instance, high prevalence of *E. dispar* (13.4%), as compared to *E. histolytica* (3.2%) and *E. moshkovskii* (1%) was recorded using single-round PCR assay from three different districts of Western Malaysia state, namely as Gerik (Perak), Jelebu (Negeri Sembilan) and Temerloh (Pahang).[10] In a recent study, Ngui et al.[11] also reported *E. dispar* to be the predominant species (26.5%) as compared to *E. histolytica* (20.4%) and *E. moshkovskii* (20.4%) among Malaysian aborigine communities in Selangor.

However, findings from this study contradict reports from several local studies where *E. histolytica* was the highest reported species in humans. A higher prevalence rate of *E. histolytica* infection was recorded in a study conducted by Ngui et al.[12] using nested PCR among 426 participants Orang Asli from five rural villages located in Pos Iskandar (Bera, Pahang), Sungai Koyan (Cameron Highland, Pahang), Sungai Bumbun (Kuala Langat, Selangor), Bukit Serok (Rompin, Pahang) and Sungai Layau (Kota Tinggi, Johore). They reported a prevalence rate of 75% for *E. histolytica,* 30.8% *E. dispar* and 5.8% *E. moshkovskii*, and adults (23.9%) had the highest rates of infection than children (15.3%). Similarly, Lau et al.[13] reported a higher prevalence of *E. histolytica* (65.4%) was found in 80% of 65 microscopically positive samples from Orang Asli settlements in Peninsular Malaysia comprising of Pulau Carey, Hulu Yam, Hulu Langat in Selangor; Bentong (Pahang) and Kuala Kangsar in Perak. Only 13.5% were identified as *E. dispar* and none were identified as *E. moshkovskii*. A recent study among two different major tribes of Orang Asli in Selangor, Temuan (Senoi ethnicity) and Mah Meri (Proto-Malay ethnicity) communities at four villages in southern and coast of

Selangor found that infection was only 7.2% (9/186) from *E. histolytica* whereas 2.2% (2/186) were from *E. dispar*.

In the current study, the prevalence of *E. histolytica* and *E. dispar* infections were not significantly correlated with neither the age groups nor gender of participants which is consistent with other published studies.[10,15,16] However, most situations have shown that younger children are more susceptible to the *E. histolytica* infection. The reason for such observation can be attributed to the weak immune system and hygiene-related behaviour such as playing in stool contaminated soil or water.[17-19]

In addition, working status, low educational level, and the absence of toilets in the household are also been identified in the current study related to *Entamoeba* spp. infection, although not statistically significant. Based on the survey, the majority of them worked as rubber tappers and look for forest products for a living. There is a possibility of cysts of *Entamoeba* spp. from the ground contaminated with stool was deposited on the foot of participants when walking along the way to the forest, which could explain the source of transmission. This finding is in parallel with a study conducted by Nath et al.[20], who found a significant association of *E. histolytica* with employment status, in which the infection was increased two-fold among daily labourers among the Northeast Indian population.

The present study also observed the participant's level of education exhibited association with *E. histolytica* infection, though not statistically significant. Probably, non-educated participants would have less knowledge and awareness about parasitic infections compared to educated individuals. Similar findings have been reported in previous studies from different countries including Saudi Arabia, Nigeria, and Ethiopia.[21-24] A study conducted in Yemen also showed that a low educational level was 1.75 times greater risk of being infected with pathogenic *E. histolytica*.[25]

Moreover, our finding showed that participants who had a safe water supply (tap water) are less likely to be infected with *Entamoeba* spp. infection. Even though a safe water supply was provided in their house, most of the participants still used an unsafe source of water from rivers and streams for drinking, bathing, and washing. This could contribute to the higher possibility to be infected with *Entamoeba* spp. In contrast, a recent study in Yemen and other reports in Brazil and Turkey found that using unsafe drinking water was a risk factor of *Entamoeba* spp. and other intestinal parasitic infections.[25-27] The present study also observed the absence of a toilet in the house was contributed to higher infection of *E. dispar* and *E. histolytica* among participants. Thus, they tend to defecate around the house and it is common practice in the communities. Our findings are in agreement with Al-Areeqi et al.[25], who found the absence of toilet in house demonstrated a 2.89 greater risk of contracting *E. dispar* infection.

The present study has identified different associated risk factors of *E. histolytica* infection related to sanitary practices. The risk factors were negligence to the practice of washing hands after contact with soil or gardening and the habit of indiscriminate defecation. Based on our observation, individuals who neglect the practice of washing their hands after contact with soil or gardening were at 3.9 times greater risk of being infected with pathogenic *E. histolytica*. Similar findings were observed by Rinne et al.[28], Alyousefi et al.[29] and Duc et al.[30] who showed that the transmission risk of *E. histolytica* increased more than two-fold if hands are not properly washed after exposure to soil or gardening. A study conducted in the suburban and urban area of western Malaysia also showed that this practice contributed 4.7 times greater risk of being infected with *E. histolytica*.[10]

The present study also highlighted that indiscriminate defecation was more likely to be infected with *E. histolytica* compared to those having access to flush latrine. Based on our observation, 51% of the participants did not have proper sanitary facilities at their home, which led them to defecate discriminately around their house especially in bushes and nearby rivers. Similar reports were observed by Ngui et al.[12], Al-Mekhlafi et al.[31] and Elyana et al.[32] who found that rivers are usually used as defecation sites, especially among children of Orang Asli. Under such circumstances, this water source may be highly contaminated with cysts of *Entamoeba* spp. from human stool and potentially become the source of infection to

others. A study among rural communities in Russas, northeastern Brazil, and the Taabo area of south-central Coˆte d'Ivoire also showed parallel findings, where subjects who practice open defecation exhibited a significantly higher positive rate compared with subjects who defecate in latrines.[26,33]

In addition, having improper sewage disposal was associated with a lower risk of being infected with *E. histolytica* as compared to those having proper common drainage and this finding is consistent with a previous study conducted in Gerik (Perak), Jelebu (Negeri Sembilan), and Temerloh (Pahang).[10] In their study, individuals who practising outdoor sewage disposal were 2.5 times greater risk of being infected with non-pathogenic *E. dispar* that could be attributed to contamination of water sources with *Entamoeba* spp. and other pathogenic parasites when runoff of slurries and sewage sludge flowing into rivers. Based on our observation at these three villages, most of the households lacked household garbage bins and commonly practising outdoor sewage disposal.

In our study, we found a significant association between *E. dispar* infection with one of the gastroenteritis symptoms such as vomiting. Similar results were previously reported in Brazil who identified *E. dispar* among symptomatic patients.[34,35] These results corroborate the findings of Costa et al.[36] and Dolabella et al.[37], who showed the Brazilian strain of *E. dispar* trophozoites is potentially pathogenic and can produce amoebic liver abscess under *in vivo* conditions.[38] Thus, *E. dispar* should be considered in the diagnosis of patients presenting with gastrointestinal symptoms, especially when other pathogens such as virus and bacteria are not detected, since most asymptomatic infections found worldwide are now attributed to this non -invasive amoeba.[27,28] There were no *E. moshkovskii* positive cases found in the collected samples during this study. Our findings showed 46 samples with no amplification of *Entamoeba* spp. by PCR. To prevent false-negative detection for samples with a low intensity of DNA that fell below the PCR detection limit, a more sensitive and specific technique such as real-time PCR can further be used.

## LIMITATIONS

There were some restrictions in our methodology. Owing to the cultural believes of some Orang Asli against giving their stool, only a single stool sample was obtained, instead of the advisable three consecutive samples. Thus, this limits our ability to determine more significant associated risk factors due to the small sample size. We expected a higher prevalence of *Entamoeba* spp. infection among Orang Asli communities in the current research location especially when rivers (known as the source of *Entamoeba* infection) were used for most of their daily activities including bathing, washing household items, and improper defecate. Availability of more stool samples can further enhance detection of *Entamoeba* spp. especially *E. moshkovskii* as well the other two similar morphology species in the future among this population.

## CONCLUSION

In conclusion, this study provides valuable updated data on the distribution and associated risk factors of *Entamoeba* spp. infection among Orang Asli communities in Malaysia, particularly in the Southern region of Perak, where *E. dispar* was more prevalent than *E. histolytica*. Indiscriminate defecation, improper sewage disposal and not washing hands after playing with soil or gardening, and having gastrointestinal symptoms such as vomiting were associated with *E. histolytica* and *E. dispar* infection. Current data could aid in providing necessary treatment as well as improving the health education and environmental health conditions for Orang Asli communities in Malaysia.

## ETHICS APPROVAL

Ethical clearance was issued by the Research Ethics Committee of Universiti Pendidikan Sultan Idris (Ref. No.: 2019-0003-01) and permission for fieldwork was obtained from the Department of Orang Asli Development (JAKOA), Ministry of Rural and Regional Development Malaysia. Informed written consent was obtained from the participants for stool sampling and information gathering.

## CONFLICT OF INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

## AUTHOR's CONTRIBUTION

All authors have made contribution to this work.

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