DEPARTMENT OF ZOOLOGY STUDENT STUDY PROJECTS

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TITLE: A REVIEW ON FLUOROSIS STUDY IN TELANGANA STATE.

SUBMITTED BY

A. VASANTH
B. PRASAD
M. SOUNDARYA
J. PAVAN KALYAN
Y. MEGHANA
P. SRIJA
G. SRUJANA
G. PRIANJALI
B. ARUN KUMAR
G. SWAPNA

KAKATIYA GOVERNMENT COLLEGE HANAMKONDA, WARANGAL.

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Aims and Objectives

The aim of our research was to review the literature about fluoride toxicity and to inform physicians, dentists and public health specialists whether fluoride use is expedient and safe.

* The Objectives of are as follows: To collect, assess and use the baseline survey data of fluorosis of Ministry of Drinking Water Supply for starting the project;

* Comprehensive management of fluorosis in the selected areas;

* Capacity building for prevention, diagnosis and management of fluorosis cases.

INTRODUCTION

Fluoride occurs naturally in our environment and is always present in our lives. Exposure can occur through dietary intake, respiration and fluoride supplements. Fluoride can be toxic in extremely high concentrations. Its everyday use in concentrations present in beverages for dental hygiene is safe.Fluorosis, a public health problem is caused by excess intake of fluoride through drinking water/food products/industrial pollutants over a long period. It results in major health disorders like dental fluorosis, skeletal fluorosis and non-skeletal fluorosis.

Fluorosis continues to be a regional issue in Telangana to this day, even decades after the first cases were discovered in Telangana in 1937. More than three lakh people in the district are affected with skeletal and dental fluorosis, a stigma that has stuck for generations. Excessive fluoride intake leads to fluorosis, a chronic condition marked by mottling of the teeth and, if severe, calcification of the ligaments.

As per the WHO norms, the permissible limit of fluoride in water is one mg/litre or one ppm (one part per million), whereas, in Telangana, 1108 habitations in 59 *mandals* are affected with high fluoride concentrations of over one ppm in groundwater. Also, 484 habitations in

17 *mandals* show fluoride levels greater than 2.5 ppm. The root cause of fluorosis is the extensive development of borewells. There are around 5.5 lakh deep borewells, which have penetrated the subsurface levels where there is a high fluoride content. This has accentuated the problem further. People in Telangana have been drinking this water for decades and it is common to see individuals with ruptured bone structures and crippled youngsters.



A people's movement to demand safe fluoride-free water gathered momentum in the last few decades alongside political developments for a new Telangana state. All this culminated in two important developments. First, was the provision of surface water, mainly from Nagarjunasagar reservoir since 2005 to Telangana. The reservoir provides Krishna water, which has permissible limits of fluoride. This helped deal with the vital public health problem to a large extent. Second, a multi-department coordinated District Fluoride Monitoring Centre (DFMC) was set up in 2012. The UNICEF came forward to support this and formed a multi-partite partnership with the district administration, the National Institute of Nutrition (NIN) and the Fluoride Knowledge and Action Network (FKAN). The DFMC had been assigned with the mammoth task of monitoring the work of 17 line departments on fluorosis mitigation. Given the magnitude of surface water supply to the entire district, and the enormity of the DFMC's mandate, ranging from safe water supply, health detection, nutrition-based relief, rehabilitation, and other activities, help was sought from the FKAN in October 2013. This was to further strengthen the DFMC as an institution and to bring in innovation from other places for a long-term solution of the Telangana fluorosis problem.

The DFMC worked on understanding the status of efforts on fluoride mitigation in Telangana, filling gaps in knowledge, and then in developing future models of action in partnership with the institutions in the region. A study on reverse osmosis plants in Telangana helped look at the diverse models in action, access issues and water management practices followed. An all NGO network meeting was conducted along with the DFMC to synthesise all efforts in the district and to plan out further efforts. A specialised training on integrating fluoride issues within watershed programmes was conducted with the idea of upscaling it at the national watershed programme level.



A specific focus of the DFMC has been on the highly affected 46 villages of the Marriguda *mandal* where all departmental activities of 17 departments associated with the DFMC are being monitored. The mobile-based Caddisfly fluoride water testing was carried out in the entire Marriguda *mandal* in partnership with Engineers Without Borders (EWB). This helped develop a comprehensive fluoride map and supplement existing fluoride data from government sources.

The DFMC also helped develop people based reporting on water supply. The FKAN helped the DFMC to conduct review meetings with the district collector by following up and synthesising action taken reports from the concerned departments, preparing agenda and action plans for DFMC. It also conducted two workshops with the UNICEF, to bring together all stakeholders concerned with the fluorosis issue in Telangana and brought them towards a comprehensive approach at tackling the problem.

Engaging with Telangana fluorosis gave the FKAN useful information to tackle the problem nationally. The condition of surface water supply has improved significantly after safe water was provided on a massive scale to fluorosis affected people from Krishna and other surface water sources. Water supply gaps exist, both in terms of inaccessible areas, and non-regular supply due to infrastructure maintenance issues, but increasingly, these are being sorted out. People are going back to fluoride-affected water supply during the gaps in water supply but as many as a third of the rural population is using reverse osmosis treated drinking water. In the future, localised surface based water supply schemes need to be in place, based on local water security and watershed planning that ensures that there are no water supply gaps. This will help wean them off payment based reverse osmosis water.

As regards food and nutrition, though some provision of special ration cards is in place for people seriously affected by skeletal fluorosis, the focus is still on rice and cereals. Shifting to non-fluoride affected food and increased nutrients, especially calcium, is absent. Special programmes for women and children continue in highly affected *mandals* (17 out of 55), but this is not translating into an overall food intake change and nutritional improvement. Agriculturebased nutritional improvement programme is critical for Telangana as a combination of millets growth and consumption, local vegetable gardens, poultry, and others to tackle the food and nutrition crises on a large scale. Specific nutrient-rich food replete with calcium, vitamin C etc., which can detoxify fluoride from the body needs to be promoted and consumed.

As regards medical relief and rehabilitation, a referral system is needed with local resource centre along with key hospitals in Hyderabad to help people badly affected by fluorosis. Patients need critical health care, as a combination of safe painkillers, physiotherapy, nutritional supplementation, assistive devices, and in some cases, safe surgical procedures for corrective action. A referral medical system as a combination of both private and public healthcare needs to be in place for both immediate relief through health centre based in Telangana, and special care through centres at Hyderabad and other places. These systems need to be free or highly subsidised so that affected people can benefit.





MATERIALS AND METHODS

Telangana In Dodo Wadera village, a Telangana-based water treatment system in the Rift Valley of Ethiopia was selected in collaboration with the National Fluorosis Mitigation Project office of the Ethiopian Ministry of Water, Irrigation and Electricity as a site to evaluate the potential for enhancing the fluoride removal capacity of the Telangana system. The community level defluoridation system consists of raw water tanker, reactor tanker with a mixer shaft, treated water tanker, sludge storage tank, water distribution point and the powerhouse. The capacity of the reactor tanker (one batch) is 5000 Liters. The Telangana system uses aluminum sulfate (alum) and calcium oxide (lime) chemicals added to the reactor tanker and mixed rapidly with

high fluoride concentration water. The motor agitated mixing and reactions of chemicals are conducted for 20 min and the treated water is stored in treated water storage tanker. The aluminum and lime used in the Telangana system were purchased from Melkassa Aluminum Sulfate Production Company in Ethiopia. Based on the working manual of the Fluorosis Mitigation Project Office of the Ethiopian Ministry of Water, Irrigation and Electricity, the amount of alum and lime used to treat 5000 liters of water per batch was 5.85 and 2.93 kg, respectively (Table 1 and SI-1). The quantity of lime added was assumed to be 50% of the alum needed for the treatment which agrees with the quantity of lime recommended as 20 to 50% of the alum dosage by Dahi et al. (1996). The quantity of alum and lime recommended in this study was targeted to achieve the WHO guideline value of 1.5 mg/L. Dahi et al. (1996) indicated that an alum dosage of 12.8 and 6.4 g lime (50% of alum quantity) reduced the fluoride concentration to 2.1 ± 0.7 mg/L based on the findings from studies conducted in 76 families. Line is added to alum to maintain neutral pH since the hydrolysis of aluminum hydroxide releases H + ions and to facilitate formation of dense floc for rapid settling (Shrivastava and Rani, 2009). Both alum and lime were dissolved in separate buckets and poured into the Telangana system as slurry and stirred for 20 min until it reaches equilibration within 2 h. In this study, efforts were made to improve the performance of the existing Telangana system considering the modality of operation by the local community, that is, the quantity of alum and lime added and the duration of mixing. The second option was using a hybrid system (the existing Telangana system and adding aluminum hydro(oxide) (AO)) prepared by the National Fluorosis Mitigation Project Office, Ministry of Water, Irrigation and Electricity, Ethiopia. The third option was using the existing Telangana system and adding cow bone char powder which is a byproduct of bone char produced by Oromo Self-Help Organization (OSHO), Ethiopia. AO was prepared by adding 100g Al2(SO4)3.14 H2O in 500 mL of Deionized water (DI) and NaOH solution that gives 2.7 OH:Al ratio due to its highest performance and surface properties (Mulugeta et al., 2014). The resulting pH 2.7 was raised to neutral pH using 2 M NaOH. In this study, 4.5 kg alum, 0.75 kg lime and 250 g AO (Table 1) were mixed in a bucket and poured into the Telangana system as slurry and stirred for 15 min until it reaches equilibration within 2 h. Thermally activated cow bone (bone char) was prepared by heating cow bone in a furnace at 500°C to remove volatile and organic matters. Bone chars have been widely used as an adsorbent for removal of excess fluoride concentrations. The fluoride removal mechanisms of bone chars are direct adsorption of

fluoride on bone char surfaces and ion exchange mechanisms where fluoride ions exchange with hydroxyl ion (Equation 5) (Kawasaki et al., 2009). Bone char powder used in this study was obtained from OSHO bone charring site located in Modjo town in the Ethiopian Rift Valley. The quantity of alum, lime and cow bone char powder added to the Telangana system was 4.5 and 1.6 kg and 300 g, respectively.



The Telangana technique In the Telangana Technique two chemicals, alum (aluminium sulphate or kalium aluminium sulphate) and lime (calcium oxide) are added to and rapidly mixed with the fluoride contaminated water. Induced by a subsequent gentle stirring, "cotton wool"-like flocs develop (aluminium hydroxides) and are subject to removal by simple settling. The main contents of the fluoride is removed along with the flocs, probably due to a combination of sorption and ion exchange with some of the produced hydroxide groups. The removal process, which is still not fully understood, has by some authors been designated as a co-precipitation. The Telangana Technique has been applied in India at different levels. On household scale it is introduced in buckets or drums and at community scale in fill and draw plants. For larger communities a waterworks-like flow system is developed, where the various processes of mixing, flocculation and sedimentation are separated in different compartments (NEERI 1987). In the guidelines for household defluoridation published by NEERI in 1987, alum is to be added as a 10 per cent solution to a 40 litre bucket equipped with a tap. This was a modification of the previously described method, where alum was added as tablets (Bulusu et al 1979). The amount of chemicals required to reach 1 respectively 2 REACHING THE UNREACHED: CHALLENGES FOR THE 21ST CENTURY mg/l fluoride are presented as a function of the fluoride concentration and the alkalinity of the raw water in a dosage design table, originally

published by Nawlakhe et al. (1975). Unfortunately, the experiences gained in Tanzania and Denmark have shown that the usability of these design guidelines has two serious limitations: Many water sources have fluoride/alkalinity limits outside the ranges presented in the table. Furthermore, the recommended addition of lime, as 5 per cent of the added alum, have shown to result in pH-values in the treated water which are significantly different from what is optimum for the fluoride removal (Lagaude et al. 1988, Dahi et al. 1995). Defluoridation in the two bucket system The designed defluoridator consists of two buckets equipped with taps and a sieve on which a cotton cloth is placed as illustrated in figure 1. Alum and lime are added simultaneously to the raw water bucket where it is dissolved/suspended by stirring with a wooden paddle. The villagers are trained to stir fast while counting to 60 (1 minute) and then slowly while counting to 300 (5 minutes). The flocs formed are left for settling for about one hour. The treated water is then tapped through the cloth into the treated water bucket from where it tapped as needed for drinking and cooking. Our investigations have shown that at least some of the fluoride, which has been captured in the flocs, is released slowly back to the water. The use of two buckets should thus ensure that the treated water is separated from the fluoride containing sludge directly after the defluoridation. All physico-chemical processes are thus performed in the raw water bucket, while the treated water bucket is kept only for the storage of the defluoridated water. Both containers are 20 litre plastic buckets, supplied with covers and equipped with one tap each, 5cm above the bottom to enable trapping of sludge. This type of bucket is produced in Tanzania, robust, cheap and very common, used by almost every family in Ngurdoto for fetching and storage of water. The two small brass taps are imported from India at a low cost and can be installed by a local craftsman using a simple tool for punching the plastic. The sieve acts as an extra safety device collecting any flocs which may escape through the tap in the raw water bucket. Normally, the water is completely clear, even more clear than the raw water, because the flocculation and sedimentation also remove water turbidity

RESULTS AND DISCUSSION

The separate addition of cow bone char powder and aluminum hydro(oxide) into the existing Nalgonda system (Aluminum sulfate and lime) significantly enhanced the fluoride removal capacity. The existing Nalgonda system (alum and lime) lowered the initial fluoride concentration of 9.3 to 7.0 mg/L, which is significantly higher than the WHO guideline value of 1.5 mg/L. The treated water fluoride concentration in the reactor tanker increased to 8.0 mg/L after 20 h of treatment due to the higher dosage of lime added above the design requirement for the treatment. This situation raised the pH which resulted in competition between OHand Fand thereby reduced the fluoride removal capacity. Thus, the inappropriate ratio of alum and lime used by the local scheme operator in the Nalgonda reactor raised the pH and reduced the fluoride removal capacity of the system. The design requires the addition of a specific proportion of alum and lime (lime = 50% alum quantity). According to the field level observation of the Nalgonda system operation by local community, steel plates and bowls were used to measure the quantity of alum and lime added to Nalgonda reactor tanker. The OU WaTER Center's study conducted

in July 2014 on the operation of Dodo Wadera Nalgonda-based treatment system indicated that 7 kg alum and 5 kg of lime (71% of the alum quantity) was added by the community, which is more than the recommended lime dosage level (50% of alum). Further, since a separate tanker for treated water was not installed, the treated water stays in the treatment tank and the sludge releases OHback to the system and thereby raising the pH which reduced the fluoride removal capacity. It was observed that the Nalgonda system produced large quantity of sludge which required labor to clean it before the next round of water treatment. Up on the addition of AO to the existing Nalgonda system, initial fluoride concentration of 9.3 mg/L was reduced to 2.8 mg/L (Figure 3). The treated water samples were collected at 5 h interval after the addition of AO and analyzed for fluoride and pH. Besides the improved performance of the treatment system, the addition of AO resulted in a lower quantity of sludge produced. Cow bone char powder (BC) added to the Nalgonda system could lower the initial fluoride concentration to 2.1 mg/L (Figure 3). This study demonstrated that both AO and cow bone char powder added to the existing system could be used to enhance the fluoride removal capacity of Nalgonda techniques. The treated water fluoride concentration met the US standard for fluoride of 4.0 mg/L. However, the treated fluoride level is still slightly above the WHO guideline value of 1.5 mg/L thus requiring further optimization of chemicals dosage (alum, lime and AO) and pH to meet the standard. Further, these two media (AO and cow bone char powder) produced significantly lower quantities of sludge which was one of the common problems of the existing Nalgonda systems. The combination of the alum/AO and cow bone char powder has a beneficial effect on the properties of the treated water by reducing the quantities of alum and lime needed to treat the water. Upon the addition of AO/cow bone char powder into the Nalgonda Technique, less sludge was produced, and the treated water quality met the WHO guideline values compared to the Nalgonda Technique alone. The addition of these media into the Nalgonda Technique enhances the formation of aluminum hydroxide flocs which is responsible for the coprecipitation of the fluoride ions. Further, the combined AO/cow bone char powder and alum/lime is low-cost and the treated water is affordable to the rural communities.

Conclusion

Based on the nine entomologic investigations conducted between 2017–2019, it was concluded that *An. culicifacies* was present throughout the year while *An. fluviatilis* had seasonal presence in the study areas. *Anopheles culicifacies* was resistant to alphacypermethrin and emerging resistance to deltamethrin was observed in this area. *Anopheles culicifacies* was confirmed **as** the malaria vector. This type of information on indigenous malaria vectors and insecticide resistance is important in implementation of vector control through indoor residual spraying (IRS) and use of insecticide-impregnated bed nets for achieving the malaria elimination goals.

The State in 2021, recorded 877 cases of malaria, much lower than average of the last three years, 1157, as per the National Vector Borne Disease Control Programme. The Test positivity Rate (TPR) has fallen to 0.03 in December 2021 as compared to the previous three years' average of 0.04. In most of the months of 2021, the TPR was as low as 0.02. The disease is however restricted to the districts of Bhadradri Kothagudem and Mulugu.

There have been zero deaths due to Malaria in the State since 2017. Since then, the State has also recorded declining cases of malaria, except the marginal rise in 2021. While the number of malaria cases in 2017 was 2,688, the State only saw 1,792 cases in 2018, and 1,711 in 2019. In a big dip, the State only registered 870 cases in 2020, while 877 cases were recorded in 2021.

A team from the State malaria control programme were felicitated in New Delhi on Monday by the officials of the Ministry of Health and Family Welfare (MoFHW) on the occasion of World Malaria Day. State Health Minister Harish Rao congratulated the team and hailed the State's efforts in controlling the disease by means of better sanitation measures,

References

Bulusu, K.R., Sundaresan, B.B., Pathak, B.N., Nawlakhe, W.G. et al. Fluorides in Water, Defluoridation Methods and Their Limitations. Journal of the Institution of Engineers (India), vol. 60, p. 1-25, 1979.

Dahi, E., Orio, L., Bregnhj, H. Sorption Isotherms of Fluoride on Flocculated Alumina. In Proceedings of the 4th Workshop on Fluorosis and Defluoridation of Water, Ngurdoto, Tanzania, October 18-22 1995 (in Press).

Gitonga, J.N. Partial Defluoridation of Borehole Water. In: Flurosis Research Strategies, (ed Likimani, S.), Department of Dental Surgery, University of Nairobi, 1984.

Gumbo, F.J. Partial Defluoridation of Drinking Water in Tanzania. In: Proceedings of the Second Workshop on Domestic Water Health Standards with Emphasis on Flurode, Arusha, Tanzania, 1987

. Lagaude, A., Kirsche, C., Travi, Y. Défluoruration des Eaux Souterraines au Sénégal Travaux Préliminaires sur L'eau du Forage de Fatick. (Eng Defluoridation of ground waters in Senegal preliminary work in the case of Fatick waters). Techniques Sciénces Methodes (in French), vol. 83:9, p.449-452, 1988.

Nawlakhe, W.G., Kulkarni, D.N., Pathak, B.N., Bulusu, K.R. Defluoridation of Water by Telangana Technique Indian Journal of Environmental Health, vol. 17.1, p. 26-65, 1975.

NEERI. Defluoridation. Technology mission on drinking water in villages and related water management. National Environment Engineering Research Institute. Nagpur 440020, India. 1987

A study of malaria vector surveillance as part of the Malaria Elimination Demonstration Project in Warangal Distract

I SEMESTER STUDENTS 2016-17

1.A.SRUJANA
2.B.VIDYA
3.CH.HARIKA
4.G.SWAPNA
5.G.NARESH
6.K.MANASA
7.K.SRIKANTH
8.M.RAJESH
9.S.PRAVALIKA
10.A.AKHIL

1.3 AIMS AND OBJECTIVES

The aims and objectives of this study are:

- 1. To identify the breeding sites of mosquitoes.
- 2. To know the species of mosquitoes that are highly prevalent in Uyo urban.
- 3. To determine the physico-chemical parameters of the breeding sites.
- 4. To know their various control measures.

Methods

Telangana State has received national recognition and appreciation for its efforts to eliminate malaria in the past six years between 2015 and 2021, as part of the National Framework for Malaria Elimination in India (NFMEI) initiative of National Vector Borne Disease Control Programme (NVBDCP), Director General of Health Services (DGHS), Ministry of Health and Family Welfare (MOHFW), New Delhi

Due to the untiring efforts to eliminate malaria, the Director General of Health Services (DGHS), MOHFW, has said that Telangana, which had Category-2 classification in malaria elimination has now been upgraded and classified as Category-1, senior health officials here said.

Results & Discussion

The two known malaria vector species (Anopheles culicifacies and Anopheles *fluviatilis*) were found in the study area, which have been previously reported in this and adjoining areas of the State of Madhya Pradesh. The prevalence of An. culicifacies was significantly higher in all study villages with peak in July while lowest number was recorded in May. Proportion of vector density was observed to be low in foothill terrains. The other anopheline species viz, Anopheles subpictus, Anopheles annularis, Anopheles vagus, Anopheles splendidus, Anopheles pallidus, Anopheles nigerrimus and Anopheles barbirostris were also recorded in the study area, although their prevalence was significantly less compared to the Anculicifacies. In 2017, An. culicifacies was found to be resistant to dichlorodiphenyl-trichloroethane (DDT) and malathion, with possible resistance to alphacypermethrin and susceptible to deltamethrin. However, in 2019, the species was found to be resistant to alphacypermethrin, DDT, malathion, with possible resistance to deltamethrin. The bioassays revealed 82 to > 98% corrected % mortality of An. culicifacies on day-one post-spraying and 35 to 62% on follow-up day-30. Anopheles culicifacies sibling species C was most prevalent (38.5%) followed by A/D and E while B was least pre-dominant (11.9%). Anopheles fluviatilis sibling species T was most prevalent (74.6%) followed by U (25.4%) while species S was not recorded. One An.culicifacies (sibling species C) was found positive for Plasmodium *falciparum* by PCR tests in the mosquitoes sampled from the test areas.

removal of debris and waste, conducting dedicated programmes across Telangana

References:

1. Sharma VP. Re-emergence of malaria in India. Ind J Med Res. 1996;103:26-45.

Guerin PJ, Dhorda M, Ganguly NK, Sibley CH. Malaria control in India: A national perspective in a regional and global fight to eliminate malaria. *J Vector Borne Dis.* 2019;56:41–5. doi: 10.4103/0972-9062.257773.

3. World Health Organization. World Malaria Report 2019. Accessed December 7, 2019.

4. National Vector Borne Disease Control Programme (NVBDCP). Malaria.. Accessed October 8, 2019.

5. Dhingra N, Jha P, Sharma VP, Cohen AA, Jotkar RM, Rodriguez PS, Million Death Study Collaborators et al. Adult and child malaria mortality in India: A nationally representative mortality survey. *Lancet.* 2010;376:1768–74. doi: 10.1016/S0140-6736(10)60831-8.

6. Sharma RK, Thakor HG, Saha KB, Sonal GS, Dhariwal AC, Singh N. Malaria situation in India with special reference to tribal areas. *Indian J Med Res.* 2015;141:537–41.

7. Nema S, Verma AK, Bharti PK. Strengthening diagnosis is key to eliminating malaria in India. *Lancet Infect Dis.* 2019;19:1277–8. doi: 10.1016/S1473-3099(19)30544-4.

8. Okell LC, Ghani AC, Lyons E, Drakeley CJ. Submicroscopic infection in Plasmodium falciparum-endemic populations: a systematic review and meta-analysis. *J Infect Dis.* 2009;200:1509–17. doi: 10.1086/644781.

9. Ahmad A, Verma AK, Krishna S, Sharma A, Singh N, Bharti PK. Plasmodium falciparum glutamate dehydro-genase is genetically conserved across eight malaria endemic states of India: Exploring new avenues of malaria elimination. *PloS One*. 2019;14:e0218210. doi: 10.1371/journal.pone.0218210.

10. Singh MP, Chand SK, Saha KB, Singh N, Dhiman RC, Sabin LL. Unlicensed medical practitioners in tribal dominated rural areas of central India: Bottleneck in malaria elimination. *Malaria J.* 2020;19:18. doi: 10.1186/s12936-020-3109-z.

11. Singh J, Purohit B, Desai A, Savardekar L, Shanbag P, Kshirsagar N. Clinical manifestations, treatment, and outcome of hospitalized patients with Plasmodium vivax malaria in two Indian states: a retrospective study. *Malar Res Treat*. 2013;2013:341862.

12. Douglas NM, Anstey NM, Buffet PA, Poespoprodjo JR, Yeo TW, White NJ, Price RN. The anaemia of Plasmodium vivax malaria. *Malaria J*. 2012;11:135. doi: 10.1186/1475-2875-11-135.

13. Medicines for Malaria Venture. Children and malaria: treating and protecting the most vulnerable. Accessed December 8, 2019.

14. Kumar A, Valecha N, Jain T, Dash AP. Burden of malaria in India: Retrospective and prospective view. *Am J Trop Med Hyg.* 2007;77:69–78. doi: 10.4269/ajtmh.2007.77.69.

15. Kitojo C, Gutman JR, Chacky F, Kigadye E, Mkude S, Mandike R, et al. Estimating malaria burden among pregnant women using data from antenatal care centres in Tanzania: a population-based study. *Lancet Glob Hlth*. 2019;7:e1695–705. doi: 10.1016/S2214-109X(19)30405-X.

16. Pereira MA, Clausen TM, Pehrson C, Mao Y, Resende M, Daugaard M, et al. Placental sequestration of Plasmodium falciparum malaria parasites is mediated by the interaction between VAR2CSA and chondroitin sulfate A on syndecan-1. *PLoS Pathog.* 2016;12:e1005831. doi: 10.1371/journal.ppat.1005831.

17. Zhou SS, Zhang SS, Zhang L, Rietveld AE, Ramsay AR, Zachariah R, et al. China's 1-3-7 surveillance and response strategy for malaria elimination: Is case reporting, investigation and foci response happening according to plan? *Infect Dis Poverty*. 2015;4:55. doi: 10.1186/s40249-015-0089-2.

18. Sitohang V, Sariwati E, Fajariyani SB, Hwang D, Kurnia B, Hapsari RK, et al. Malaria elimination in Indonesia: Halfway there. *Lancet Global Hlth*. 2018;6:e604–6. doi: 10.1016/S2214-109X(18)30198-0.

19. Lal AA, Rajvanshi H, Jayswar H, Das A, Bharti PK. Malaria elimination: Using past and present experience to make malaria-free India by 2030. *J Vector Borne Dis.* 2019;56:60–5. doi: 10.4103/0972-9062.257777.

20. Hassanpour G, Mohebali M, Zeraati H, Raeisi A, Keshavarz H. Asymptomatic malaria and its challenges in the malaria elimination program in Iran: A systematic review. *J Arthropod Borne Dis.* 2017;11:172–4.

21. Cheaveau J, Mogollon DC, Mohon MA, Golassa L, Yewhalaw D, Pillai DR. Asymptomatic malaria in the clinical and public health context. *Expert Rev Anti-infect Ther*. 2019;17:997–1010. doi: 10.1080/14787210.2019.1693259.

22. Das S, Saha B, Hati AK, Roy S. Evidence of artemisinin-resistant Plasmodium falciparum malaria in eastern India. *New Engl J Med.* 2018;379:1962–4. doi: 10.1056/NEJMc1713777.

23. van der Pluijm RW, Tripura R, Hoglund RM, Phyo AP, Lek D, Ul Islam A, et al. Triple artemisinin-based combination therapies versus artemisinin-based combination therapies for uncomplicated Plasmodium falciparum malaria: a multicentre, open-label, randomised clinical trial. *Lancet.* 2020;395:1345–60. doi: 10.1016/S0140-6736(20)30552-3.

24. Takeuchi R, Lawpoolsri S, Imwong M, Kobayashi J, Kaewkungwal J, Pukrittayakamee S, et al. Directly-observed therapy (DOT) for the radical 14-day primaquine treatment of Plasmodium vivax malaria on the Thai-Myanmar border. *Malaria J*. 2010;9:308. doi: 10.1186/1475-2875-9-308.

25. Gai PP, Van Loon W, Siegert K, Wedam J, Kulkarni SS, Rasalkar R, et al. Duffy antigen receptor for chemokines gene polymorphisms and malaria in Mangaluru, India. *Malaria J*. 2019;18:328. doi: 10.1186/s12936-019-2966-9.

26. Commons RJ, Simpson JA, Thriemer K, Hossain MS, Douglas NM, Humphreys GS, et al. Risk of Plasmodium vivax parasitaemia after Plasmodium falciparum infection: a systematic review and meta-analysis. *Lancet Infect Dis.* 2019;19:91–101. doi: 10.1016/S1473-3099(18)30596-6.

27. Eisele TP, Larsen D, Steketee RW. Protective efficacy of interventions for preventing malaria mortality in children in Plasmodium falciparum endemic areas. *Int J Epidemiol.* 2010;39(suppl_1):i88–101. doi: 10.1093/ije/dyq026.

 Walldorf JA, Cohee LM, Coalson JE, Bauleni A, Nkanaunena K, Kapito-Tembo A, et al. School-age children are a reservoir of malaria infection in Malawi. *PloS One.* 2015;10:e0134061. doi: 10.1371/journal.pone.0134061.

29. Mishra AK, Bharti PK, Kareemi TI, Chand SK, Tidgam AS, Sharma RK, et al. Field evaluation of zero vector durable lining to assess its efficacy against malaria vectors and malaria transmission in tribal areas of the Balaghat district of central India. *Trans Royal Soc Trop Med Hyg.* 2019;113:623–31. doi: 10.1093/trstmh/trz046.

30. Lancet T. Vaccines: A step change in malaria prevention? *Lancet*. 2015;385:1591–3. doi: 10.1016/S0140-6736(15)60774-7.

31. Ghosh SK, Rahi M. Malaria elimination in India—the way forward. *J Vector Borne Dis.* 2019;56:32–40. doi: 10.4103/0972-9062.257771.

32. Doolan DL, Hoffman SL. DNA-based vaccines against malaria: status and promise of the multi-stage malaria DNA vaccine operation. *Int J Parasitol.* 2001;31:753–62. doi: 10.1016/S0020-7519(01)00184-9.