Post Flood Chemoprophylaxis with Doxycycline for Leptospirosis in an Endemic Area; Case Control Study

Jayakrishnan Thayyil[1], Swathi Vipinan Koramboor[2], Athira Thottathan[3]

[1]Professor, Department of Community Medicine, Government Medical College, Calicut
[2]Junior Resident, Department of Community Medicine, Government Medical College, Calicut
[3]Junior Resident, Department of Community Medicine, Government Medical College, Calicut

ABSTRACT

Introduction: During the year 2019, Kerala state experienced flooding during August. Following this mass chemoprophylaxis with doxycycline was given in the flood in affected areas. In this context, the study was conducted with the objectives to identify the risk factors of leptospirosis and to know the usefulness of chemoprophylaxis with doxycycline in an endemic area.

Methodology: It was conducted at a public sector tertiary care hospital setting. Cases were adult patients admitted in medical ward with confirmed diagnosis of leptospirosis and the controls were selected from the bystanders of the selected cases. Collected data were entered into MS Excel and analyzed using PASW'18 software.

Results: Among cases (n=38), 84.2% were males and the age was 43.87 (15.5) years. Among controls (n=47), 85.1% were males with age of 45.83 (14.17) years. Out of this, occupational exposure (OR 4.22, 95% CI 1.61 – 11.09, p=0.003) and any wound during past one month (OR 27.6, 95% CI 3.49.17 – 217.75,p=0.001) were significantly associated with incidence of leptospirosis. Intake of doxycycline within 2 weeks of flood was found to be protective (95% OR 0.09, 95% CI 0.01-0.72, p=0.023).

Conclusion: Our study found that occupational factors, and any wound or injury are important risk factors of leptospirosis and prophylaxis with doxycycline immediately after flood can reduce the risk of leptospirosis.

Keywords: Leptospirosis, Human Risk factors , Chemoprophylaxis, Doxycycline, Post flood

INTRODUCTION

Leptospirosis is an important public health problem associated with significant morbidity and mortality. The yearly incidence of leptospirosis is in the range of 0.1 to 1 per 100 000 in temperate climates, 10 to 100 per 100 000 in the tropics, and more than 100 per 100 000 during outbreaks. [1]The magnitude of the problem in the coastal states of India is largely attributed to climatic and environmental conditions. But in view of changing agro economic conditions, leptospirosis is increasingly being reported from different parts of India.[2] The outbreaks of leptospirosis have been reported from coastal states of India including Kerala where seasonal out beaks have been reported since 1989[3]and it became endemic with peaks during monsoon and post monsoon months it was included as a notifiable disease.[4,5]

Leptospirosis is usually a seasonal disease that starts at the onset of the rainy season and declines as the rains recede. Frequent outbreaks occur in different parts of the country mostly following heavy rain falls and consequent flooding. [2] From Kerala, every year more than 1000 cases were reported in with case fatality rate of around 10% and the cases are showing an increasing trend from the year 2015[6]. Compared

to the previous years with average (<1400), an increase in number of cases (2200) and deaths were reported in 2018, which was attributed due to heavy rain fall and floods. The peak was observed in the month of September, following floods during July and August in 2018. [6] The national guide lines advice to use Doxycycline 200 mg, once a week, as chemoprophylaxis to high-risk persons in areas where clustering of cases has been reported during the peak transmission season. [2] During the year 2019, the state experienced flooding during the second and third week of August. Considering the above guide lines and with the previous years' experience of surge of leptospirosis following flood, the department of health and family welfare of the state have taken advance measures to give mass chemoprophylaxis by widely distributing doxycycline for weekly consumption to public in the flood affected areas [2,7]. Except in high risks groups during out breaks, there were no conclusive evidences available for mass chemoprophylaxis among public and also, reported results from different parts of the world were contradictory [7,8,9] There is no conclusive evidence in the literature recommending usefulness of chemoprophylaxis as a massive level public intervention or for the use of low-risk exposure after a deluge.[8,9]

Prevention and control of leptospirosis require an understanding of risk factors. Leptospirosis infection is acquired through contact of abraded skin and/or mucus membrane with the environment contaminated with urine of rodents, carrier or diseased animals. Risk factors for leptospirosis can be classified into three main groups: animal factors, environmental factors, and human factors. [1,2, ,9,10] Therefore, information on risk factors for leptospirosis disease or infection is a key requirement for successful leptospirosis prevention and control programs. [11] In tropical countries, the basic determinants of leptospiral transmission namely, presence of carrier animals, suitable environment for the survival of leptospira and behavioural and occupational factors of people that predispose them to get infection are common. But, the magnitude and nature of these factors vary from community to community. Therefore, the specific risk factors for acquiring leptospiral infection could be unique to each community. [9] An understanding of the transmission cycle of the disease in the community and the modifiable risk behaviours is essential for planning strategies for prevention and control.

In this context, the study was conducted with the following objectives; first, to identify the risk factors of leptospirosis during an outbreak and second, to know the usefulness of chemoprophylaxis with doxycycline to reduce the risks during an outbreak following flood.

METHODS

The study was conducted at a public sector tertiary care hospital setting in northern part of Kerala where about 30% of the leptospirosis cases from the state were reported in the preceding years. The district is known endemic for leptospirosis. The period of study was from 1st September to 30th September 2019 which was two weeks following the flood in August.

Subject selection: *Eligibility criteria for cases and controls-* Cases were adult patients admitted in medical ward with confirmed diagnosis of leptospirosis either by ELISA or PCR.[2,6] They were selected consecutively from wards according to their willingness and eligibility criteria. The controls were selected from among the bystanders of the selected cases who are either their relatives or friends in the neighbourhood. They are selected as age (±5) and sex matched in the ratio of 1:1. Patients and their by standers who were in serious condition and admitted in the ICUs, were excluded from selection.

The information was collected by the investigators directly by conducting face to face interview at the bed side with the subjects using a pre tested structured schedule. Along with socio-demographic variables, the following exposure variables within last four weeks were collected; Human behavioural factors like occupation, use of personal protective measures, any injury or skin aberrations, contact with animals and exposure to stagnant water, animal risk factors like presence of domestic animals and contact with them and rat menace, environmental risk factors like exposure to flood, long time contact stagnant water etc. Chemoprophylaxis details like history of intake and timing of chemoprophylaxis with Doxycycline following flood were also collected. The outcome variable was hospital admission with clinical symptoms and diagnosis of leptospirosis.

During the study period, the subjects were enrolled consecutively till we got the adequate sample size. So, there was no selection bias. The selection of controls was done simultaneously, based on their health status; those who have got any clinical symptoms suspecting leptospirosis, present or past history of fever during last two months or any past history of leptospirosis were excluded. The data collected were cross checked for consistency.

Study size calculation was done by epitools (www. epitools.ausvet.com.au/case control). With an expected proportion of exposed in controls groups as 0.05% and assumed Odds ratio (OR) of 7.5 with confidence interval (CI) of 0.95 and desired power of 0.8, the required sample size was 37.

The selection and data collection were done only after getting informed consent from subject. This was conducted as public health activity under Regional Prevention of Epidemic and Infectious Disease cell (R-PEID cell) of Government of Kerala as part of operational research on emergency basis and so was exempted from ethical review.

Statistical Analysis: Data collected were entered into MS Excel and analyzed using PASW'18 software.

Qualitative data were expressed as percentages and quantitative data as measures of central tendency. Association of various risk factors with leptospirosis infection were assessed by uni and bivariate analysis using appropriate statistical tools like Odds ratio was calculated with 95% confidence intervals.

RESULTS

During our study period of one month, total 58 adult patients with suspected leptospirosis was admitted in medical wards of which 46 were confirmed by laboratory criteria. Out of this 40 who satisfied our inclusion criteria were enrolled as cases and data were collected. Due to data discrepancy two cases were excluded, so finally 38 were included as cases and analysis was done. The controls were selected from the close relatives who were staying in the same households of cases. In most cases, the bystanders were persons from the same family, so there may be overmatching. So, to overcome these issues, instead of 1:1 ratio, we increased the size of controls by selecting one additional bystander as control who is not a member of same household. Finally, we have recruited 47 controls and were included in analysis. Since the investigators collected the data directly there was no missing of data.

Table 1: Measurement of Association of Risk factors with cases and controls

Variable	Cases (n=38)	Control (n=47)	OR	95% CI	P value
Demography					
Mean age(year)	43.87± 15.15	45.83± 14.17	NA	NA	0.834
Gender Male	32 (84.21)	40 (85.10)	0.70	(0.23 – 3.05)	0.909
Environmental Risks					
Flood surrounding House	20 (52.6%)	23 (48.9%)	1.16	(0.49-2.73)	0.734
Human Risks					
Occupational exposure present	19 (50%)	9(19.14%)	4.22*	(1.61-11.09)	0.003*
Long time Contact with water or soil	33 (86.8%)	34 (72.3%)	2.5	(0.81 – 7.9)	0.110
Wound /Injury present	18 (47.4%)	01 (2.1%)	27.6*	(3.50- 217.75)	0.001*
Personnel Protective measures	34 (89.5%)	45 (95.74%)	*0.37*	(0.06 -2.18	0.276
Animal Risks					
Domestic Animal contact	01 (2.6%)	02 (4.2%)	0.60	(0.05-6.97)	0.690
Existence of Rat	13 (34.2%)	13 (27.7%)	1.36	(0.54-3.43)	0.515
Chemoprophylaxis					
Doxy intake within 2 weeks	01 (2.6%)	11 (23.40%)	0.09*	(0.011-0.72)	0.023*

NB: *Significant p value <0.05; OR= Odds Ratio; CI= Confidence Interval

Demography: Among cases (n=38), sex wise 32 (84.2%) were males. The mean (SD) age was 43.87 (15.5) years and most of them were from lower socioeconomic groups. Among controls (n=47), sex wise 40 (85.1%) were males with mean (SD) age of 43.87 (15.5) years (Table 1).

The results of the risks factors are given under headings: human behavioural, animal, and environmental risk factors as mentioned earlier.[10,11]

Human behavioural risk factors: Among cases, 19 (50%) belonged to occupational risk category, 34 (89.8%) used some sort of personal protective measures while working. This was 19 (50%) and 45 (95.7%) respectively among controls.

Among cases, 18(47.4%) had any injury or skin aberrations during last one month period, only one had any contact with animals, and 33 (80.6%) had contact with either stagnant water or soil for long time. Among control this was 1, 2(4.2) and 34(72.3%) respectively.

Animal risk factors: Among cases and controls, 13 (34.29), 2(4.2%) respectively had existence of domestic animals, rat menace at their premises.

Environmental risk factors: Among cases, 20 (52.6%) were affected with flood or stagnant water surrounding their house, while among controls it was 23 (48.9%).

Prophylaxis: Among cases, only one person had taken doxycycline as prophylaxis within 2 weeks of flood, while among controls 11 (23.40%) person have taken doxycycline as prophylaxis within 2 weeks of flood.

The details of prevalence of risk factors in cases and controls, association of risk factors and their strength of associations (Odds ratio with 95% CI) are given in the table 1. Out of this risk factors, occupational exposure (OR 4.22, CI 1.61 – 11.09, p=0.003) and any wound during past one month (OR 27.6, 95% CI 3.49.17 – 217.75, p=0.001) were significantly associated with incidence of leptospirosis. Intake of doxycycline within 2 weeks of flood was found to be protective (95% OR 0.09, CI 0.01-0.72, p=0.023).

DISCUSSION

We have conducted a case control study in a tertiary care hospital setting by taking (n=38) confirmed cases of leptospirosis with age, sex matched controls (n=47) who were bystanders of cases either residing in the same household or neighbours or relatives from the same geographical area. Since the incubation period of leptospirosis ranged from 5 days to 20

days, the study was conducted after an interval of 14 days following flood.[2,11] During the year 2019, the flood occurred during month of August third week. With the previous experience of surge of cases following deluge, the department of Health have started "mass prophylaxis campaign" against leptospirosis, by door to door distribution of doxycycline to the public in the flood affected areas through field workers. Along with these activities "Doxycycline distributing booths "were opened at all public places and public hospitals [6]. As per RPEID cell record in the previous three years (2016:557, 2017:838, 2018:670) patients had under gone in patient treatment from our centre.

Gender: Since leptospirosis is related with outdoor occupational activity, majority (84.2%) of the patients were males. It was similarly reported else were in the country.[4,5,10,13] A previous work from the centre reported 58.9% were men.[13]

Age: Leptospirosis usually affect people of productive age group. Our cases' age was 43.8+15 years. Since among the productive age group, the older persons are engaged in agricultural activities, that was the reason for skewing to older age. [2,6] A previous study from Andaman Islands reported that it affected young age groups of age 23.8+11 years. [16]

Human factors: Occupation- Leptospirosis was associated with occupational activities like farming, agricultural activities; our cases also had occupational relationship. Work related to contaminated surface water like paddy field or other agricultural activities are related to leptospirosis. [5,14,15,16] Our study has found that occupation related to exposure to contaminated water have high risk for leptospirosis (OR 4.22, CI 1.61 – 11.09, p=0.003). Earlier one observational descriptive study from the area reported no significant association with occupational risk factors.[13]

Injury: The transmission path of organism is through abraded skin or mucosa. Existence of a wound in the lower limb was found to be an associated risk factor in 10 out of 16 studies [17]. In an earlier study from here, 62.9% had wounds on the feet .[13] Our study found out that any injury, fissuring, ulcer in the lower limb has got a high chance of getting leptospirosis (OR 27.6, 95% CI 3.49 – 217.75, p=0.001). Since wound is a modifiable risk factor through which bacteria can enter in to body, it should be considered as an important component of control programs. Either such persons should avoid to enter in the water-logged area or use of water proof adhesive plasters and antibiotics should be advised. [2,6]

Contact with stagnant water: Stagnant water surrounding the house associated with leptospirosis was reported in 7 out of 12 studies,[17] stagnant water may be contaminated with rats or animals' urine containing Leptospira which may survive there for weeks. While standing wet water the skin may get soften for easy penetration of bacteria as entry point.

[14,16] In the present study, compared to controls (72.3%) cases were more (86.8%) exposed to long time contact (more than 1 hour with stagnant water but the risk was not statistically significant. (OR 2.9, 95% CI 0.85 -9.95).

Personal protective measures: The personal protective measures include wearing footwears, shoes, long clothes, gloves while doing job or washing with soap and water immediately after the work. [1,2] This association was reported in a review paper in 5 out of 9 studies. [16,17] In our study also the controls have used any of the personal protective measures more but, the risk reduction was not significant (OR 0.37, 95% CI 0.06 – 2.18).

Animal or rat associated risk factors. The domestic animals like cattle, sheep, dogs may harbour leptospirosis and their urine may contain bacteria.[11] Another study from India found the presence of cattle in the house have high odds OR 5.1. [16] A study from the state reported 29.6% of rats have evidence of past leptospirosis infection[5]. Similarly, the presence of rats at the premises may excrete urine along with bacteria. This was similarly reported in many other studies. [4,5,18,19,20] In our study, very few of cases or controls (2.4% vs 4.2%) have domestic animals with them and the findings were insignificant. Compared to controls (27.7%), more cases (34.2%) have noticed presence of rats at their home but the risk was not significant (OR 1.36, 95% CI 0.54- 3.43)

Flood: Leptospirosis is associated with rain. During flood, there may be stagnation water which may contain bacteria from soil, contaminated animals' urine. Also, during flooding, the rats may escape from its burrows and get caught in flood and may move towards human dwelling for shelter.[9,11] Thus, there may be chances for increased human-animal as well as human-pathogen contact. Flooding during the past 14 days was found to be a risk factor in a previous study from the same district, as well as in 6 out of 10 studies from other countries. [17,21] Compared to controls (48.9%), more cases (52.6%) reported that flood have surrounded their house or work place, but the risk of exposure was not significant (OR 1.16 95% CI 0.49 – 2.73). This was similarly reported from north India.[8,22]

Doxycycline prophylaxis: The use of doxycycline as chemoprophylaxis was first reported in 1980s among US army with relative risk of (RR) 4.2Vs 0.2%. [23] Another evidence from India following flood was from an ecological study reported from Bombay based on historical data.[8] In the absence of big RCTs there was no robust evidence for supporting this and after that many conflicting reports are published. [7] A study early conducted in India reported that in endemic area, doxycycline will not prevent infection but has got significant protective effect on morbidity, mortality. [24] Another study reported that the effect depends upon a number of factors like high risk of exposure, timing and coverage. [25] A non RCT conducted at Thailand after flood found that it was pro-

tective in reducing morbidity (76%), it was increased in case of laceration,[26] and at the same time another RCT conducted at Iran comparing with azithromycin, placebo reported that it has no effect in reducing clinical morbidity and compared with azithromycin, it was less effective in reducing infection.[27]

In our study, it was found that compared to controls (11- 23.4%), among cases, only one (2.6%) had taken any doses of doxycycline within two weeks of flood proving that it will reduce the risk (OR 0.09, 95% CI 0.1- 0.72,p=0.023) of clinical symptoms, morbidity and hospital admission. Since we did not conduct any serological study, we could not give any statement about protection offered by it for infection. In leptospirosis, most of the infection will be sub clinical,[5] and our study was conducted in hospital so, our study could not prove its effect in subclinical infections.

The present study found that out of the risk factors, the human factors like occupational exposure and any wound during past one month was significantly associated with incidence of leptospirosis. Both these are modifiable using adequate protective measures, so stress should be given to these control measures.

Intake of Doxycycline within 2 weeks of flood was found to be protective (OR 0.09, CI 0.01-0.72). Though the field workers have distributed doxycycline to the household members immediately after flood, the intake was very less even in the control group. This pointed out the fact that the drug distribution campaign should be accompanied by intense information, education, communication (IEC) activities. As some previous studies pointed out the timing of taking prophylaxis within the incubation period, within 2 weeks following flood is important. [8,16,24] Because in our study, we found that many weeks after the flood / few days before morbidity many of cases have taken doxycycline which have no effect. Similarly, this study was conducted in a highly endemic area, in post flood situation, so we could not predict replicability of our findings in non-endemic areas in non-flood situations.

While conducting community level RCTs is not feasible especially for preventive drugs, rapidly conducted case control studies can give fast results. In areas where there is paucity of data, based on the assumption that leptospirosis risk factors are relatively stable at the time of exposure and the measurement of outcome, it is acceptable to treat finding of case control studies as reliable as RCT.

LIMITATIONS

Our study was conducted in tertiary hospital settings where only patients with only serious symptoms were admitted, most of them belongs to low social class. For generalisability, the study should be conducted in community settings by selecting cases and controls from source population. The study was conducted in a single centre, single settings with few numbers of cases and controls. For proving efficacy of weekly intake of doxycycline for preventing leptospirosis morbidity and mortality, large scale RCT may be needed.

CONCLUSION

Our study concludes that occupational factors, and any wound or injury are important risk factors of leptospirosis and immediate prophylaxis with doxycycline immediately after flood can reduce the risk of leptospirosis in endemic area. Since leptospirosis is a zoonotic disease of public health importance causing huge morbidity and mortality in Asian countries, case control studies with large sample size from multiple sites of the country may be needed to build more robust evidences on this subject.

REFERENCES

1. World Health Organization. (2003). Human leptospirosis: guidance for diagnosis, surveillance and control. World Health Organization. https://apps.who.int/iris/handle/10665/42667. [Accessed May 5th 2021]

2. National Guidelines Diagnosis, Case Management Prevention and Control of Leptospirosis National Centre For Disease Control, (Directorate General Of Health Services)22-Sham Nath Marg, Delhi - 110 054. 2015.

3. Swapna RN, Tuteja U, Nair L, Sudarsana J. Seroprevalence of leptospirosis in high risk groups in Calicut, North Kerala, India. Indian J Med Microbiol.2006 Oct;24(4):349-52

4. John TJ, Rajappan K, Arjunan KK. Communicable diseases monitored by Disease Surveillance in Kottayam district, Kerala state, India. Indian J Med Res 2004; 120 : 86-93.

5. Kuriakose M, Paul R, Joseph MR, Sugathan S, Sudha TN. Leptospirosis in a midland rural area of Kerala State. Indian J Med Res. 2008 Sep;128(3):307-12. PMID: 19052343.

6. Integrated Disease Surveillance Project Directorate of Health Services Office. Thiruvananthapuram. Available from: https://dhs.kerala.gov.in/wp-content/uploads/2020/03/cddata_07052019.pdf

7. Charan J, Saxena D, Mulla S. Prophylaxis and treatment for leptospirosis: where are the evidences? National Journal of Physiology, Pharmacy and Pharmacology. 2012 Jul 1;2(2):78.

8. Supe A, Khetarpal M, Naik S, Keskar P. Leptospirosis following heavy rains in 2017 in Mumbai: Report of large-scale community chemoprophylaxis. The National medical journal of India. 2018 Jan 1;31(1):19.

9. Brett-Major DM, Lipnick RJ. Antibiotic prophylaxis for leptospirosis. Cochrane Database of Systematic Reviews 2009, Is-

sue3. Art. No.: CD007342. DOI: 10.1002/14651858.CD007342. pub2

10. Sugunan AP. Epidemiology of leptospirosis. In: World Health Organization, ed. Leptospirosis: Laboratory Manual. New Delhi, India: World Health Organization; 2007:13-21.

11. Faine S, Adler B, Bolin C, Perolat P. Leptospira and Leptospirosis. 2nd ed. Melbourne, Australia: MediSci; 2000 State Surveillance Unit.

12. Epitools. Available at epitools.ausvet.com.au/case control. [Accessed 28 Aug 2019]

13. Pappachan MJ, Mathew S, Aravindan KP, Khader AY, Bharghavan PV, Kareem MM, Tuteja U, Shukla J, Batra HV. Risk factors for mortality in patients with leptospirosis during an epidemic in northern Kerala. Natl Med J India. 2004 Sep 1;17(5):240-.

14. Tangkanakul W, Tharmaphornpil P, Plikaytis BD, et al. Risk factors associated with leptospirosis in northeastern Thailand, 1998. Am J Trop Med Hyg. 2000;63:204-208.

15. Murhekar MV, Sugunan AP, Vijayachari P, Sharma S, Sehgal SC. Risk factors in the transmission of leptospiral infection. Indian J Med Res. 1998;107:218-223.

16. Sugunan AP, Vijayachari P, Sharma S, et al. Risk factors associated with leptospirosis during an outbreak in Middle Andaman, India. Indian J Med Res. 2009;130:67-73.

17. Sakundarno M, Bertolatti D, Maycock B, Spickett J, Dhaliwal S. Risk Factors for Leptospirosis Infection in Humans and Implications for Public Health Intervention in Indonesia and the Asia-Pacific Region. Asia Pacific Journal of Public Health. 2014;26(1):15-32. doi:10.1177/1010539513498768

18. Leal-Castellanos CB, Garcia-Suarez R, Gonzalez-Figueroa E, Fuentes-Allen JL, Escobedo-De La Pena J. Risk factors and the prevalence of leptospirosis infection in a rural community of Chiapas, Mexico. Epidemiology & Infection. 2003 Dec; 131(3): 1149-56.

19. Sehgal S, Sugunan A, Vijayachari P. Leptospirosis disease burden estimation and surveillance networking in India. Southeast Asian J Trop Med Public Health. 2003;34(suppl 2):170-177.

20. Sasaki DM, Pang L, Minette HP, et al. Active surveillance and risk factors for leptospirosis in Hawaii. Am J Trop Med Hyg. 1993;48:35-43.

21. Pappachan MJ, Sheela M, Aravindan KP. Relation of rainfall pattern and epidemic leptospirosis in the Indian state of Kerala. J Epidemiol Community Health 2004; 58 : 1054.

22. Bhardwaj P, Kosambiya JK, Desai VK. A case control study to explore the risk factors for acquisition of leptospirosis in Surat city, after flood. Indian J Med Sci. 2008;62:431-438

23. Takafuji ET, Kirkpatrick JW, Miller RN, Karwacki JJ, Kelley PW, Gray MR, McNeill KM, Timboe HL, Kane RE, Sanchez JL. An efficacy trial of doxycycline chemoprophylaxis against leptospirosis. N Engl J Med. 1984 Feb 23; 310(8): 497-500. doi: 10. 1056/NEJM198402233100805. PMID: 6363930.

24. Sehgal SC, Sugunan AP, Murhekar MV, Sharma S, Vijayachari P. Randomized controlled trial of doxycycline prophylaxis against leptospirosis in an endemic area. Int J Antimicrob Agents. 2000 Feb;13(4):249-55. doi: 10.1016/s0924-8579 (99)00134-x. PMID: 10755239.

25. Schneider MC, Velasco-Hernandez J, Min KD, Leonel DG, Baca-Carrasco D, Gompper ME, et al. The use of chemoprophylaxis after floods to reduce the occurrence and impact of leptospirosis outbreaks. Int J Environ Res Public Health 2017;14:594.]

26. Chusri S, McNeil EB, Hortiwakul T, Charernmak B, Sritrairatchai S, Santimaleeworagun W, Pattharachayakul S, Suksanan P, Thaisomboonsuk B, Jarman RG. Single dosage of doxycycline for prophylaxis against leptospiral infection and leptospirosis during urban flooding in southern Thailand: a non-randomized controlled trial. J Infect Chemother. 2014 Nov;20(11):709-15. doi: 10.1016/j.jiac.2014.07.016. Epub 2014 Sep 22. PMID: 25172777.

27. Alikhani A, Salehifar E, Zameni F, Rafiei A, Yazdani-Charati J, Delavaryan L, Akbari A, Babamahmoudi F. Comparison of azithromycin vs doxycycline prophylaxis in leptospirosis, a randomized double blind placebo-controlled trial. The Journal of Infection in Developing Countries. 2018 Nov 30;12(11): 991-5.