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Characteristics of Rural Leptospirosis Patients Admitted to Referral Hospitals during the 2008 Leptospirosis Outbreak in Sri Lanka: Implications for Developing Public Health Control Measures

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Abstract. To determine the exposure risk factors of highly endemic rural leptospirosis in tropical setting, we conducted a prospective, hospital-based case control study in Sri Lanka. A conceptual hierarchy of variables was used to analyze the data. Case patients included 38 (34%) females and 73 (66%) males with a mean age of 36 yr (SD 12.7 yr). Using piped, chlorinated water for drinking/general purposes (odds ratio [OR] 0.33, 95% confidence interval [CI] 0.16–0.67), paddy fields in the vicinity of home (OR 1.77, 95% CI 1.06–2.97), sighting dogs at home yard/dog ownership (OR 1.79, 95% CI 1.11–2.91), sighting cattle at home yard/cattle ownership (OR 1.69, 95% CI 1.00–2.84), and work in a paddy field (OR 3.02, 95% CI 1.68, 5.41) were the main predictors of leptospirosis among febrile patients. In high endemic tropical settings with rural leptospirosis, risk factors in residential environments, rather than individual exposures, seemed to play a major role in leptospirosis disease transmission.

INTRODUCTION

Leptospirosis is a globally widespread zoonotic disease,[1] caused by spirochetes from the genus *Leptospira*. Human leptospirosis is a neglected condition and often overlooked, as a result of the ubiquitous nature of the illness, and the lack of resources for its diagnosis.[2] Human leptospirosis mostly affects people in tropical and subtropical countries, where the environmental conditions and socio-cultural practices are favorable for disease transmission, and also the resources for diagnosis and treatment are scarce. The estimated median global incidence of endemic human leptospirosis, is 5 cases per 100,000 population, but in high endemic settings the incidence is as high as 975 cases per 100,000.[3]

The disease transmission cycle of leptospirosis depends on its zoonotic nature: the causative spirochetes persistently colonize the renal tubules of a mammalian reservoir host, which are excreted into fresh water environments where accidental hosts such as humans contract infection.[4] The genus *Leptospira* contains 22 species,[5] of which 14 infectious species contain more than 250 serovars. A wide range of animals can transmit *Leptospira* including almost domestic, peri-domestic, and wild animals.[6] Once shed into the environment, *Leptospira* can survive in warm, wet soil, or water sources for weeks to months. In tropical countries, direct human contact with environmental sources of leptospirosis is common in both rural and urban settings. In countries where leptospirosis is not endemic, clustering of cases are often caused by point source epidemics,[7–9] and hence, epidemiological investigations are not difficult. In countries where the disease is endemic, multiple factors determine disease occurrence, with varying effects on disease transmission, making it difficult for source identification and hence, control activities. Though common exposures are reported in most descriptive types of studies, determining risk or protective factors require highly discriminatory, analytical study designs. Analyses of exposure risk factors of leptospirosis have been attempted, using case control methodology in several studies in global literature.[10–19] However, one major limitation of previous case control approaches to investigate risk factors, leptospirosis is the assumption of the same level of postulated effects on disease transmission by varying types of determinants.

Previously, we proposed a conceptual hierarchy of determinants to study leptospirosis and in this model, hypothesized that the different categories of variables could have different effects on determining disease transmission mediated through other parameters.[20] The socio-demographic and economic factors were categorized as distal variables; residential environment and factors facilitating disease transmission were considered as intermediate factors; and exposure variables were thought to be the most proximal factors that determine leptospirosis transmission (Figure 1). We proposed that the effect of distal variables are through intermediate and proximal variables, and lower level variables could not have confounding effects on upper level variables.

Sri Lanka is a tropical country where sustained outbreaks of leptospirosis have been observed since 2008.[21] The annual case incidence of leptospirosis, as passively reported to the epidemiology unit of Sri Lanka from 2008 to 2011, was estimated at 22.5 per 100,000 population, one of the highest reported incidence rates in the global literature.[22] The economy of Sri Lanka is largely based on agriculture. High seasonal rainfall, high temperature, socio-cultural practices, and residential environment, mostly in rural areas, combined to drive optimal conditions for leptospirosis transmission. Although a number of descriptive studies and ecological studies have been carried out, exposure and other risk factors of leptospirosis in Sri Lanka have not been deeply evaluated using the previously described analytical study designs. Here, we are reporting the results of a case control study based on a proposed model of conceptual hierarchy of variables for leptospirosis disease transmission in Sri Lanka in the context of ongoing outbreaks and transmission.[20]

METHODS

This case control study was carried out from September 2008 to January 2009, in three tertiary care hospitals from

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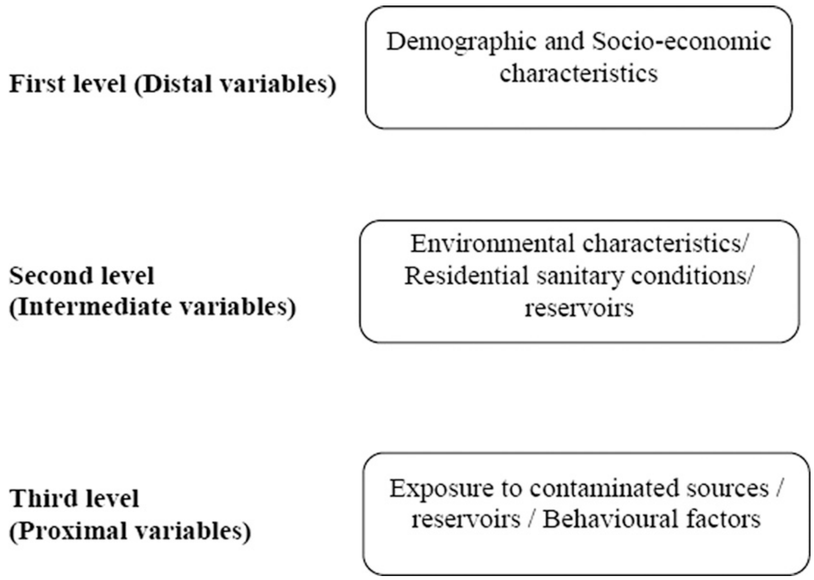

FIGURE 1. Conceptual hierarchy of variables proposed for analysis of determinants of leptospirosis.

three contrasting leptospirosis-endemic districts in central Sri Lanka. All fever patients admitted to the selected hospitals were screened using a clinical criteria checklist to select all "possible cases of leptospirosis." Confirmed cases from the clinical study were recruited as cases for this case control study. Details of patient selection criteria, diagnosis, case confirmation, serology based on the microscopic agglutination test (MAT) and clinical profile of the case population,[22,23] and the protocol for case-control study has been detailed.[20]

A confirmed case of leptospirosis was defined as a patient who presented to one of the three selected hospitals in Matale, Kandy, or Kegalle with acute febrile illness and with any of the following: 1) sero-conversion or 4-fold rise in titer between acute and convalescent phase samples based on the MAT; 2) a probable case of leptospirosis (a patient with fever, myalgia, headache, and prostration with one of the following signs or symptoms; jaundice, conjunctival suffusion, hemorrhage, oliguria or anuria, meningeal irritation, cardiac arrhythmia or failure) with single high *Leptospira* MAT titer greater than or equal to 1/800; or 3) a positive polymerase chain reaction (PCR)/real-time PCR (qPCR) test.

As a result of the observations made on the high sensitivity but imperfect specificity (ambiguity) of the clinical case definition, we selected only confirmed cases for the case control analysis. Presumptive/probable cases (clinical leptospirosis with enzyme-linked immunosorbent assay [ELISA] positive results) were excluded from the present analysis. Patients admitted to the same wards as the case patients with acute febrile illness not confirmed as having clinical leptospirosis and with a negative leptospirosis ELISA immunoglobulin M (IgM) were selected as the controls. We excluded all febrile cases that had a slight (as opposed to strongly suspected) pretest likelihood of leptospirosis. Selection of controls from the same ward allowed matching for geographical area and for assessing risk factors for disease acquisition within versus outside the house, because the study was conducted in three districts, none with overlapping hospital catchment areas. Data collection were carried out using a previously tested interviewer-administered questionnaire. Two physicians carried out the data collection.

The questionnaire included socio-demographic, residential, environmental, and exposure variables, listed according to a hierarchical framework of determinants. Distal variables, included socio-demographic factors that could influence exposure to probable sources of *Leptospira*. Intermediate determinants of leptospirosis were defined as the availability of environmental sources suitable for survival of *Leptospira* and the presence of likely reservoir host animals known to harbor the spirochete. Data on all probable environments that facilitate leptospirosis transmission within a radius of 300 meters of subjects' residences were collected. Exposure to probable environmental sources of leptospirosis were defined as the proximal variables. Exposure data were assessed for a period of 3 weeks before the appearance of first symptoms. During the univariable analysis we used the χ^2 test to describe data. Multivariable analysis was done using the proposed hierarchical model (Figure 1) for determinants of leptospirosis, using a conditional logistic regression model. All distal level variables were adjusted for each other (parallel level only); intermediate level variables were adjusted for parallel; distal and proximal variables were adjusted for all other variables. Effect modifications and interactions of parallel variables were also carried out.

This study was reviewed by the Board of Study in Community Medicine, Post Graduate Institute of Medicine, Colombo, Sri Lanka. Ethical clearance for the study was obtained from the Ethics Review Committee of the Faculty of Medicine, University of Peradeniya, Kandy, Sri Lanka.

RESULTS

Selection of cases and controls. During the study period, 401 probable cases of leptospirosis were investigated, of which 112 cases were confirmed. Of confirmed cases, 51 were positive by MAT, 22 were positive by PCR, and 39 were positive by both tests. Of the 112 definitive cases, exposure and clinical details were missing for one case, yielding 111 definitive cases for this analysis. These included 38 (34%) females and 73 (66%) males. After eliminating leptospirosis

TABLE 1
Socio-demographic characteristics of cases and controls

	Cases (N = 111) n	%	Controls (N = 233) n	%	Significance
Ethnicity					
Sinhala	103	92.8	211	90.6	$\chi2 = 0.472$
Moor/Malay*	3	2.7	6	2.6	df = 1
Sri Lankan Tamil*	2	1.8	8	3.4	$P = 0.492$
Indian Tamil*	3	2.7	8	3.4	
Residence					
Urban	2	1.8	15	6.4	$\chi2 = 3.440$
Rural*	107	96.4	216	92.7	df = 1
Estate*†	2	1.8	2	0.9	$P = 0.064$
Level of education					
Primary	21	18.9	47	20.2	$\chi2 = 2.877$
Post primary	51	45.9	119	51.1	df = 3
Secondary	37	33.3	59	25.3	$P = 0.411$
Tertiary	2	1.8	8	3.4	
Employment					
Professionals/ proprietors/managers	5	4.5	13	5.6	$\chi2 = 3.139$
Clerks/service personals	8	7.2	19	8.2	df = 4
Skilled manual workers	30	27.0	72	30.9	$P = 0.535$
Unskilled manual workers	23	20.7	57	24.5	
Unemployed	45	40.5	72	30.9	

*These categories were collapsed to a single category for significance testing.
†Estate sector included patients who are residing within tea estates of Sri Lanka. This is a completely different socio-cultural setting compared with other two areas.

IgM ELISA positive febrile patients, 222 controls were selected from 339 fever patients (these were the febrile patients after excluding all possible cases of leptospirosis) who were admitted to the study hospitals during the same study period. The mean age of the cases was 36.2 years (SD 12.7), compared with 38.6 years (SD 13.8) of the controls ($t = 1.544$, $P = 0.124$). Socio-demographic characteristics were not significantly different between cases and controls (Table 1).

Exposure to probable environmental sources. Exposure to probable environmental sources (duration and frequencies) were examined separately for all cases for a period of 3 weeks before the onset of the illness. Of the 111 cases studied, 55 (50%) had a single type of exposure attributable to the present condition. Another 48 (43%) had multiple types of exposures making the assessment of the exact point of exposure difficult. Eight patients (7%) could not recall any occasions that they might have had any probable exposures including outdoor work (Table 2).

Determinants of human leptospirosis. In the unadjusted analysis, (Table 3), using a mainline water (piped and chlorinated water) source had a 0.33 times lower risk of contracting leptospirosis, as compared with patients who were using surface water sources (wells, tanks, streams). Cases were more likely to live in an environment where there were running water sources, (odds ration [OR] 1.73, 95% confidence interval [CI] 1.07–2.80), bushes/forests, (OR 1.62, 95% CI 1.02–2.59), and paddy fields, (OR 2.17, 95% CI 1.33–3.52), compared with the residential environments of the controls. Furthermore, cases were more likely to be living in an environment where they often saw dogs (OR 1.71, 95% CI 1.08–2.70) or cattle (OR 1.69, 95% CI 1.04–2.74) in their home yards. Most cases had multiple exposures during the period under study. None of the exposure factors were significant in this initial analysis except for paddy field work. Working in paddy fields was the most common type of exposure, reported among both cases and controls. Seventy percent of the cases had worked in a paddy field during the 3-week period before the onset of their illness compared with only 38% among controls.

Exposure in the paddy fields. The most common exposure risk factor reported was working in the paddy fields. This exposure was further analyzed to explore the details of the paddy field exposure. The frequency and duration of exposure in paddy fields, showed no difference between cases and controls. Mean duration of days worked during the 3-week period before the onset of disease was 9e; median number of hours worked in a day was 5; and the median total exposure period was 31 hours for both cases and controls. However, cases who reported paddy field work were more likely to work infrequently in paddy fields. Of the 109 cases who reported exposure in paddy fields, 49 (45%) reported having had regular exposure, whereas among controls, regular paddy field exposure was reported by 64% (79 of 123). This observed difference was statistically significant (χ^2 = 8.68, $P = 0.003$). Mean total duration of regular paddy field work among controls (11.3 years/SD 7.6) was higher than that of cases (16.8 years/SD 10.1). This difference was not statistically significant ($t = 1.42$, degrees of freedom [df] = 124, $P = 0.159$).

Adjusted ORs and multivariable analysis. Multivariable analysis of determinants of leptospirosis using three-step logistic regression modeling (Table 4) (see Methods) revealed the following as determinants of leptospirosis in the study population: using mainline water for drinking/other purposes (OR 0.33, 95% CI 0.16–0.67; paddy fields in the vicinity of home (OR 1.77, 95% CI for OR 1.06–3.00); seeing dogs at home yard/dog ownership (OR 1.80, 95% CI for OR1.11–2.91), seeing cattle at home yard/cattle ownership (OR 1.69, 95% CI for OR 0.00–2.84), and work in a paddy field (OR 3.02, 95% CI for OR 1.68, 5.41).

TABLE 2
Exposure to probable sources of infection during 3 weeks before onset of illness among 111 confirmed cases of leptospirosis from three selected districts in Sri Lanka, during 2008 outbreak

	n	%
Cases with exposure to single source*		
Worked in a paddy field	46	41.4
Exposed to surface water source	6	5.4
Other outdoor work	3	2.8
Cases with exposure to multiple sources		
With a paddy field exposure	40	36.0
Without a paddy field exposure	8	7.2
Cases without a exposure history	8	7.2
Total	111	100.0

*Multiple exposure to same source.

DISCUSSION

In this hospital-based case control study, a large number of variables, grouped into three hierarchical levels, were assessed, and in the multivariable model, five variables showed independent association with leptospirosis. In the leptospirosis field, rigorous statistical separation of proximal and distal causal risk factors for leptospirosis has not previously been carried out, to our knowledge. This novel approach provides especially valuable insights into leptospirosis disease transmission mechanisms in Sri Lanka and is gener. These modifiable determinants of human leptospirosis, could be used in leptospirosis control activities. However, results of

TABLE 3

Unadjusted odds ratios for leptospirosis infection among 111 confirmed cases of leptospirosis from three selected district in Sri Lanka, during 2008 outbreak

	Cases (*N* = 111) n	%	Controls (*N* = 222) n	%	OR	95% CI for OR		*P*
Socio-demographic characteristics								
Sinhalese ethnicity	103	92.8	211	90.6	0.745	0.321	1.730	0.493
Urban residence	2	1.8	15	6.4	0.267	0.060	1.187	0.083
Secondary/tertiary education	40	36.0	70	30.0	1.312	0.813	2.116	0.266
Unemployment	45	40.5	72	30.9	1.525	0.953	2.439	0.079
Living in a permanent housing structure	94	84.7	187	80.3	1.360	0.740	2.501	0.172
Dumping solid waste at own backyard	49	44.1	85	36.5	1.376	0.869	2.185	0.174
Use of mainline (treated) water	10	9.0	54	23.2	0.328	0.160	0.673	0.002*
Residential environment (availability of these environmental sources within 200 meters of residence)								
Streams or surface water sources	77	69.4	132	56.7	1.733	1.073	2.799	0.025*
Marshy lands	29	26.1	46	19.7	1.438	0.844	2.448	0.181
Bushes/forest	72	64.9	124	53.2	1.623	1.017	2.589	0.042*
Paddy fields	79	71.2	124	53.2	2.170	1.337	3.523	0.002*
Other agricultural fields	12	10.8	26	11.2	0.965	0.468	1.992	0.923
Animal farms	8	7.2	9	3.9	1.933	0.725	5.153	0.188
Reservoirs in residential environment†								
Sighting rats at home	62	55.9	128	54.9	1.038	0.659	1.636	0.873
Sighting cats at home/cat ownership	39	35.1	66	28.3	1.371	0.846	2.221	0.201
Sighting dogs at home/dog ownership	57	51.4	89	38.2	1.708	1.082	2.696	0.022*
Sighting cattle at home yard/cattle ownership	41	36.9	60	25.8	1.689	1.040	2.742	0.034*
Sighting pigs at home yard/pig ownership	14	12.6	26	11.2	1.149	0.575	2.298	0.694
Sighting goats at home yard/goat ownership	14	12.6	20	8.6	1.537	0.745	3.170	0.244
Sighting other rodents at home yard	42	37.8	73	31.3	1.334	0.831	2.141	0.232
Exposures during 3 weeks before present illness‡								
Worked in a marshy land	17	15.3	39	16.7	1.112	0.598	2.068	0.738
Worked in a paddy field	86	77.5	114	48.9	3.591	2.147	6.004	< 0.001*
Other outdoor works	7	6.3	13	5.6	1.258	0.556	2.845	0.581
Exposed to stagnant water	5	4.5	19	8.2	0.531	0.193	1.462	0.221
Worked in a agricultural field	9	8.1	28	12.0	0.646	0.294	1.420	0.277
Worked in a bush	15	13.5	31	13.3	1.018	0.525	1.975	0.958
Exposed to running water	25	22.5	52	22.3	1.012	0.589	1.732	0.966
Handled a cow/buffalo	7	6.3	16	6.9	0.913	0.364	2.287	0.846
Handled a dog	18	16.2	22	9.4	1.856	0.951	3.624	0.528

*Significant at 0.05 level.
†Sighting animals were defined as sighting listed animals more than once a week in home yard or animal ownership.
‡Even a single exposure during a 3-week period was included as an "exposure.".
OR = odds ratio; CI = confidence interval.

the study should be interpreted with the awareness of the limitations of this study.

Because this study was hospital-based, patient selection bias was difficult to eliminate in this study. Patients attending these tertiary care hospitals may be different from those who are attending lower level hospitals in the district. Hence, the results of this study are valid mainly for the more ill patients who present to tertiary hospitals. Furthermore, cases admitted to these hospitals are usually those patients with moderate to severe disease, hence the identified determinants are more applicable to groups of patients that have moderate to severe disease. Despite the instructions given to medical officers admitting patients, it is possible that more patients with paddy field exposure may have been admitted because it is highly suspected among local clinicians that such exposure may be an important risk factor for leptospirosis. This potential but unmeasured bias might have led to an overestimation of final effect size of paddy field exposure. Interviewer bias could have also played a role in detailing the history. In this study, recall bias should be minimal because of selection of controls who were also having other diseases. However, among patients with a typical leptospirosis clinical syndrome, history-taking

TABLE 4

Adjusted odds ratios for the variables included in the final predictor model for leptospirosis among 111 confirmed cases of leptospirosis from three selected districts in Sri Lanka, during 2008 outbreak

	OR	95% CI for OR		*P*
Using mainline water for drinking/other purposes*	0.325	0.159	0.673	0.002
Paddy fields in the vicinity of home†	1.773	1.062	2.974	0.029
Sighting dogs at home yard/dog ownership†	1.796	1.107	2.914	0.018
Sighting cattle at home yard/cattle ownership†	1.687	1.002	2.841	0.049
Worked in a paddy field‡	3.019	1.684	5.410	< 0.001

*Distal variable adjusted only for parallel variables.
†Intermediate variables adjusted for all parallel and distal variables.
‡Proximal variables adjusted for all parallel, intermediate, and distal variables.
OR = odds ratio; CI = confidence interval.

procedures by admission officers and house officers, which would definitely include questions on exposures, may have influenced the answers given to data collectors, introducing systematic bias in recalling exposures. In addition, exclusion of probable cases from the study, and positive IgM ELISA patients from the control group may also have had an effect on the final effect size. Another limitation is that the study was carried out during the paddy harvesting period, where we usually see a clear peak of cases every year and in 2008, these cases are from a huge outbreak. The risk factors for endemic leptospirosis may be slightly different from these observed risk factors. All of these possible biases should be considered in interpreting our results.

The most common and strongest exposure risk factor associated with leptospirosis was observed for rice farming activities. This individual-level exposure factor was the only one identified in the multivariate analysis and strengthens the findings of previous descriptive reports from Sri Lanka. The natural focus of leptospirosis in paddy fields and the epizootic process of leptospirosis transmission have been studied widely.[24] However, in this study, when the occupational categories are considered, only 24 cases (15.5%), were reported as professional rice farmers. Another 15% reported they were involved in farming activities frequently but not as the main occupation. The rest of the exposed patients were in the paddy fields infrequently or for the first time. Among the controls, 64% of patients who reported paddy field exposure were regularly exposed. The occupation of rice farming was not found to be associated with leptospirosis. This conclusion is consistent with the results of the Ramachandran study published in 1974, in which 85.2% of the cases were engaged in off-time agricultural work and farmers accounted for only 8.6% of the study sample.[25] Studies conducted by Walloopillai and others in 1966[26] and Rajasuriya and others in 1964[27] also showed similar results. There may be biological and ecological explanations for this observation. Traditional or professional farmers who had frequent exposure might already have serovar-specific immunity. Furthermore, in Sri Lanka, traditional farmers usually have large, well-maintained fields and are working regularly for long periods. Maintenance of these fields and keeping fields free of rats is more common among these farmers. However, in-depth studies are needed to investigate these ecological factors.

Cattle ownership/sighting cattle at home yard showed a risk association and has also been reported by other authors.[28] As shown in our serological analysis of these cases, 30% of them showed highest titer against serogroups Sejroe and Hebdomadis, which are mainly found in cattle.([29–32] Cattle-borne infections would be even possible for cases who reported paddy field exposure. In Sri Lanka, between monsoon periods, after harvesting the paddy, it is common to have buffaloes and cows in these fields. *Leptospira* excreted by these animals can remain in the paddy field for months and can lead to human infection. Unlike the dry areas of the country, in the study areas, paddy fields remain as wet lands between cultivation periods and provide an ideal environment for *Leptospira* species to persist in the environment. Dog ownership/sighting dogs at home yard showed an independent risk association in our study. This is a common observation in some countries and the dog is often reported as a reservoir animal for human leptospirosis.[33–37]

Using mainline water for drinking and other purposes served as an independent protective factor associated with leptospirosis, with an OR of 0.325. This finding is compatible with previous studies, where they have reported a strong protective association of indoor water sources and leptospirosis. *Leptospira* are susceptible to chlorine and can survive for only 3 minutes in a concentration of 0.3 ppm.[6] During the chlorination procedure the residual chlorine concentration is around 5 ppm. This finding shows that rural residents, who do not have access to purified water, were more at risk of acquiring leptospirosis. One alternative explanation for this observation is that this observation may be a proxy of "urbanization" that leads to reducing the exposure factors. Although this is possible, the study setting is primarily rural and we included other proxy measures of urbanization under "residential environment," which were not significant in this analysis.

An interesting observation in this study is that the residential environmental level risk factors emerged as important variables and the only exposure variable identified was working in a paddy field. As a country where the exposure rates are very high in rural areas, these results may show why Sri Lanka has failed in controlling leptospirosis. This also shows that strategies targeting prevention of individual exposures may not be effective in controlling endemic rural leptospirosis. Community-level strategies, including improvement of living conditions and sanitation, could have profound effects in the control of this disease. These types of strategies should also have an impact on the control of other major infectious diseases including dengue, which has become a major public health menace to the country during the last decade.

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