Sir,

Immunity against diphtheria depends on the presence of antibodies against the toxin produced by natural infection or vaccination. In India, diphtheria vaccination is included in the universal immunization programme, but reports of outbreaks and persistence of toxigenic Corynebacterium diphtheriae have raised concern about its adequate coverage. Several diphtheria outbreaks have been reported in different districts of Assam since 2010 with case fatality rate up to 30 per cent. In a study by Phukan et al, it was found that the immunization coverage in Assam was limited to 62.2 per cent only.

Here, we report three cases of diphtheria during the month of September and October 2015, involving cases from three adjoining tea gardens in Dibrugarh district of upper Assam. Of the three index cases, two (male: 1; female: 1) were admitted in a tertiary care centre in Dibrugarh district and the third case (male) was admitted to a private hospital. All three were in the 15-18 yr age group, and presented with pain in the throat, difficulty in swallowing and fever. Of the three cases, two expired. Both these patients were tested positive for C. diphtheriae by laboratory investigation at the respective hospitals. None of the three cases had vaccination cards nor records were found in the tea garden hospitals and public health centres (PHCs).

Following request from the State Health authority to the Regional Medical Research Centre of the Indian Council of Medical Research, Dibrugarh, Assam, a rapid investigation with reference to the index cases was done in the respective areas in October 2015. These areas were inhabited by tea garden workers mainly engaged in tea industry. All close contacts were investigated so as to ensure proper treatment. The male:female ratio of the surveyed population was 2:3. Throat swabs were collected from household members of the positive cases available (n=12) and also the neighbouring households (n=149) by house-to-house survey. The house of the index case and all the households in the same line and the adjacent two lines were visited for sample collection. In tea gardens, a line represents a cluster of 10-20 households. The Table shows the number of samples collected from the different gardens and number found positive for C. diphtheriae by culture. Throat swab culture was carried out in the bacteriology laboratory of RMRC, Dibrugarh by direct inoculation on blood agar and tellurite blood agar plates and incubation at 37° C for 24-48 h. C. diphtheriae were identified based on colony morphology on tellurite blood agar, presence of metachromatic granules, catalase test, urease test and sugar fermentation tests. On tellurite blood agar the colonies appeared as black colonies. On Albert’s staining these appeared as green coloured bacilli with bluish black metachromatic granules at one or both ends. The isolates were catalase positive, urease negative and fermented glucose and maltose. The vaccination history was uncertain as no vaccination cards were available with them nor the records were found in the tea garden hospitals and PHCs.

The demographic data of 10 culture-positive cases revealed that most (n=7) of them were females and were in the 20-28 yr age group (n=8). However, one was a nine year old boy and another was a 60 yr old female. Of these positive cases, two were family members and the rest were neighbours of the index cases. Except the 9 yr old child, none had any symptoms. The child presented with a sore throat and low-grade fever and was referred to a health facility for further management. Among the 10 contacts, nine did not have records of vaccination...
except the 9 yr old boy who had received only a single dose of DPT vaccine (as per documentary evidence).

Thus, most of the culture positives were adults between 20 and 28 yr of age. In the previous outbreak investigations also, maximum cases were in the adult age group. The sensitivity testing was carried out using Kirby Bauer disc diffusion method on Mueller Hinton agar with 5 per cent sheep blood following CLSI guidelines and interpretative criteria as that for Staphylococcus aureus. The sensitivity pattern of the isolates showed that of the 10 isolates, four were sensitive and six were intermediately sensitive to erythromycin while all (100%) were sensitive to azithromycin, ciprofloxacin, gentamicin, ceftaxime and tetracycline. None of the isolate was sensitive to penicillin.

In conclusion, there was evidence of circulating C. diphtheriae strain in the community with the possibility of repeated outbreaks in vulnerable persons. Therefore, community-based screening of antibody titre to understand vaccination status against C. diphtheriae is necessary to undertake appropriate preventive measures.

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Conflicts of Interest: None.

**Table.** Samples collected and found positive for Corynebacterium diphtheriae from different tea gardens

| Tea garden | Index case (n) | Number of family members of the index cases | Number of family members positive for C. diphtheriae | Number of non-family members | Number of non-family members positive for C. diphtheriae | Total number of throat swabs collected | Total positive for C. diphtheriae |
|------------|---------------|--------------------------------------------|---------------------------------------------|--------------------------------|---------------------------------------------|---------------------------------------|----------------------------------|
| Garden I   | 1             | 3                                          | 1                                           | 26                             | 0                                           | 29                                     | 1                                |
| Garden II  | 2             | 4                                          | 0                                           | 36                             | 1                                           | 40                                     | 1                                |
| Garden III | 3             | 5                                          | 1                                           | 87                             | 7                                           | 92                                     | 8                                |
| Total      | 3             | 12                                         | 2                                           | 149                            | 8                                           | 161                                    | 10                               |

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