Diphtheria: A zoonotic disease in France?

Isabelle Bonmarin\textsuperscript{a,\*}, Nicole Guiso\textsuperscript{b}, Anne Le Flèche-Matéos\textsuperscript{b}, Olivier Patey\textsuperscript{c}, A.D. Grimont\textsuperscript{b}, Patrick Grimont\textsuperscript{b}, Daniel Levy-Bruhl\textsuperscript{a}

\textsuperscript{a} Institut de veille sanitaire, 12 rue du Val d’Osne, 94415 St Maurice, France
\textsuperscript{b} Institut Pasteur, Centre National de Référence des corynébactéries toxinogènes, Paris, France
\textsuperscript{c} Centre hospitalier, Villeneuve St Georges, France

\begin{abstract}
Thanks to vaccination, diphtheria has almost disappeared in France. The case definition, used for mandatory notification, was expanded in 2003 to include toxin-producing strains of \textit{Corynebacterium ulcerans}. We describe the epidemiology of diphtheria in France from 1990 to 2008. No cases occurred between 1990 and 2001. Since 2002, 19 cases have been reported: 4 cases due to \textit{Corynebacterium diphtheriae} related to exposure in endemic countries, and 15 cases due to other corynebacteria, including 4 cases of pseudomembranous pharyngitis, mainly related to contact with domestic animals. High vaccination coverage in the population and sensitive surveillance need to be maintained. Moreover, control measures need to be adapted to the non-\textit{C. diphtheriae} toxigenic species.
\end{abstract}

\section{1. Introduction}

Universal diphtheria vaccination and mandatory notification were introduced in France in the 1930s. The French current diphtheria immunization schedule consists of three doses given at 2, 3 and 4 months of age, followed by a booster given at 15–18 months. Subsequent boosters are administered every 5 years up to 18 years. Since 2005, decennial adult boosters have been recommended. The very high and sustained coverage achieved in infants (98\%) has led to a dramatic decrease in incidence, from around 45 000 cases after the Second World War to fewer than five cases per year in the 1980s. In 2003, notification criteria were expanded to include toxin-producing strains of \textit{Corynebacterium ulcerans}, as now recommended by the European Centre for Disease Prevention and Control (ECDC). We describe the epidemiology of diphtheria since the last indigenous case due to \textit{Corynebacterium diphtheriae}, reported in 1989, and the consequences of broadening the case definition.

\section{2. Methods}

As soon as a case of diphtheria is suspected or a toxin-producing strain is detected in humans, the local public health authority (Ddass) and the National Institute for Public Health Surveillance (InVS) must be alerted, to allow immediate implementation of control measures. The clinician or microbiologist in charge of the patient or swab specimens sends a standardized reporting form to the Ddass, which is then forwarded to national level at the InVS. The information collected includes demographic data, a brief clinical history, microbiological characteristics, disease outcome, vaccination status and control measures implemented. Since 1990, all forms have been entered in an Epi Info database. We describe the information collected through the notification forms, including any available notes, letters and published or unpublished investigation reports [1,2].

The microbiological analyses are performed by the peripheral hospital microbiology laboratories and the National Reference Laboratory (NRL) at the Institut Pasteur (Paris) which is part of the Diphtheria Surveillance Network (DIPNET) [1]. All isolates are sent to the NRL who performs their identification using gram staining, colony observation, API coryne strips (API-bioMérieux, La Balme les Grottes, France), PCR detection of the diphtheria toxin gene [2], and antibiotic resistance according to the recommendations of the French Society for Microbiology [3]. Between 2002 and 2007, no test to verify toxin expression was performed. The Elek test was added only in 2008 but all the isolates presented in the current analysis were tested retrospectively. Between 2002 and 2006, the isolates were ribotyped both manually (with manual DNA extraction) and using the RiboPrinter directly from agar culture [4]. Ribotyping also provides species identification (PvuII ribotypes to differentiate \textit{C. diphtheriae} from the non-\textit{C. diphtheriae}, and BstEII ribotypes to distinguish \textit{C. ulcerans} from \textit{Corynebacterium pseudotuberculosis}) and typing (mostly with BstEII). Since 2008, we have introduced Rosco test (Eurobio, Les Ulis, France) and Hiss Serum Water Sugars to
confirm the glycogen data of the API coryne strips. We have also introduced the PCR targeting pld gene [5] to better distinguish C. pseudotuberculosis and C. ulcerans. We are not using rp08 sequencing [6] since it does not differentiate these two species.

To assess the completeness of the surveillance system, the notification database was matched with the one from the NRL, which contains data on toxigenic Corynebacterium clinical isolates received since the NRL was nominated in 1990.

3. Results

No toxigenic isolate was identified by the NRL between 1990 and 2001. Since 2002, 18 diphtheria cases with isolates harbouring the tox gene in their genome have been reported through the statutory notification system and have been identified by the NRL. An additional case identified by the NRL was not reported to the InVS, giving a total of 19 cases: 4 cases were due to C. diphtheriae, 12 to C. ulcerans and 3 to C. pseudotuberculosis. Although not explicitly included in the new case definition, cases due to C. pseudotuberculosis have been integrated in the analysis (Fig. 1).

3.1. C. diphtheriae cases

The four cases due to C. diphtheriae were seen in two women, a child and a man. A 26-year-old woman from China, who had been living in France for 2 years and was unvaccinated, became ill with pseudomembranous pharyngitis. The source of infection was not identified. She was staying in France illegally and had very limited contact with people outside her home. However, she probably had contacts with other immigrants from China. She was hospitalized in an intensive care unit (ICU) because she presented with renal failure and QT interval prolongation the day after onset of the disease. She received antitoxin serum a few days later. A 5-year-old boy, who had been fully immunized for his age, presented with non-severe pharyngitis after returning from Madagascar. He was to receive his fifth dose of vaccine 4 months later. A 45-year-old man was injured in a traffic accident in Madagascar, and the bacterium was identified from a wound on his leg 2 weeks later. He had received a booster dose more than 20 years before. A 71-year-old woman, returning from a trip to Russia, became ill with pseudomembranous pharyngitis and was hospitalized for 4 months. All four cases recovered fully.

All C. diphtheriae isolates were producing diphtheria toxin.

3.2. Cases due to other Corynebacterium spp

The 15 cases not due to C. diphtheriae were seen in nine women and six men, aged between 28 and 82 years, median age 63 (Table 1). There were four cases of pseudomembranous pharyngitis, all in women (all C. ulcerans), eight skin ulcers (six C. ulcerans and two C. pseudotuberculosis), one otorrhea in a woman hospitalized for stroke (C. ulcerans), one lymph node suppuration (C. pseudotuberculosis) and one bacteraemia (C. ulcerans). Seven of the fifteen patients were hospitalized. Two women infected with C. ulcerans presented with neurological symptoms. None received serotherapy since diphtheria antitoxin has not been available in France since 2003. The first case was admitted with a velopharyngeal paralysis which extended to the hypopharynx. She was fed for 3 months with parenteral nutrition, and developed several food aspiration pneumonias. One month after admission, she developed diphtheric polyneuritis [7]. The other woman with C. ulcerans had neurological toxigenic symptoms 2 weeks after onset of the disease. No deaths occurred.

Eight of the fifteen cases infected with C. ulcerans or pseudotuberculosis had predisposing factors:

- Immunosuppressive treatment after kidney transplantation in a 47-year-old woman with pseudomembranous pharyngitis, admitted to ICU.
- Chronic renal failure and dialysis in a 55-year-old man with cutaneous ulceration on his leg.
- Poor hygiene conditions in an 80-year-old man with a varicose ulcer.
- Type 2 diabetes, well-controlled by diet, in a 69-year-old woman with pseudomembranous pharyngitis.
- Diabetes in three men with skin ulcers.
- Leukaemia in a 46-year-old woman with C. ulcerans bacteraemia (two positive blood cultures).

Diphtheria immunization status was known for only six cases: two of them had not been immunized and four had received more than three doses of diphtheria vaccine (a 53-year-old who had received a final booster more than 30 years before, a 46-year-old woman with bacteraemia who had received a booster 1 year previously, and a 28-year-old diabetic man with skin ulcer who had received a booster 1 year before. The date of last booster was not available for the fourth case).

None of the 12 patients with C. ulcerans had a history of recent travel or contact with dairy animals but 10 did have contact with domestic animals (Table 1). Two animals were found to be positive for C. ulcerans and one for Corynebacterium auriscanis. The patients in contact with these dogs developed pseudomembranous pharyngitis (n = 2) and otorrhoea (n = 1) due to C. ulcerans. Automated ribotyping of the isolates indicated that C. ulcerans isolates from the patients and their respective dogs were indistinguishable. One patient had contact with a sick cat, which was tested negative. Three other animals were not tested because the owners could not afford to pay the veterinary fees and the public animal health authority refused to sample the dogs because diphtheria is not considered to be a zoonotic disease. One other dog died before it could be tested. To our knowledge, only one positive dog was tested a second time, following treatment with amoxicillin: the bacterium was still present and the animal was euthanized.

Of the three cases due to C. pseudotuberculosis, one patient lived in a rural environment, but had no direct contact with two goats belonging to a neighbour or with a herd of Australian angora goats living 800 m from the house. These animals were not tested. Another case was in a patient who did not have any contact with animals. Information was unavailable for the third case.

The identification of C. pseudotuberculosis and C. ulcerans, based on the data of the API coryne strips, were confirmed by the use of the Hiss serum test, except for one of the C. ulcerans collected in 2008 from a cutaneous lesion. In this last case, the API coryne strips identified reproducibly C. pseudotuberculosis and the Hiss serum reproducibly C. ulcerans. The pld PCR data are ambiguous and develop-

![Fig. 1. Diphtheria cases, 1975–2008, according to Corynebacterium toxigenic species, France.](image)
**Table 1**

<table>
<thead>
<tr>
<th>Year</th>
<th>Sex</th>
<th>Age</th>
<th>Toxigenic symptoms</th>
<th>Clinical description</th>
<th>Antitoxin therapy</th>
<th>Vaccinated</th>
<th>Species* (and expression of toxin according to the Elek test)</th>
<th>Contact with animals</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>2002</td>
<td>F</td>
<td>26</td>
<td>Renal + cardiac</td>
<td>Pseudomembranous pharyngitis</td>
<td>Yes</td>
<td>No</td>
<td><em>C. diphtheriae</em> (Elek+)</td>
<td>Dog tested</td>
<td>Chinese</td>
</tr>
<tr>
<td>2003</td>
<td>M</td>
<td>82</td>
<td>Cutaneous lesion</td>
<td><em>C. ulcerans</em> (Elek−)</td>
<td></td>
<td></td>
<td><em>C. ulcerans</em> (Elek−)</td>
<td>Positive dog</td>
<td>Immunodeficient</td>
</tr>
<tr>
<td>2003</td>
<td>F</td>
<td>47</td>
<td>Cutaneous lesion</td>
<td><em>C. ulcerans</em> (Elek+)</td>
<td>Yes</td>
<td>No</td>
<td><em>C. ulcerans</em> (Elek−)</td>
<td>Dog not tested</td>
<td>Poor hygiene conditions**</td>
</tr>
<tr>
<td>2004</td>
<td>F</td>
<td>69</td>
<td>Neurologic</td>
<td>Pseudomembranous pharyngitis</td>
<td></td>
<td></td>
<td><em>C. pseudotuberculosis</em> (Elek+)</td>
<td>No animal</td>
<td>Diabetic</td>
</tr>
<tr>
<td>2004</td>
<td>F</td>
<td>5</td>
<td>Pharyngitis</td>
<td><em>C. diphtheriae</em> (Elek+)</td>
<td></td>
<td>Last &lt; 5 years</td>
<td><em>C. pseudotuberculosis</em> (Elek−)</td>
<td>Negative dog</td>
<td>Chronic renal failure</td>
</tr>
<tr>
<td>2004</td>
<td>M</td>
<td>55</td>
<td>Cutaneous lesion</td>
<td><em>C. ulcerans</em> (Elek+)</td>
<td></td>
<td></td>
<td><em>C. ulcerans</em> (Elek−)</td>
<td>Negative dog</td>
<td>Chronic renal failure</td>
</tr>
<tr>
<td>2005</td>
<td>F</td>
<td>79</td>
<td>Cutaneous lesion</td>
<td><em>C. ulcerans</em> (Elek+)</td>
<td></td>
<td></td>
<td><em>C. pseudotuberculosis</em> (Elek+)</td>
<td>Dog not tested</td>
<td>Chronic renal failure</td>
</tr>
<tr>
<td>2006</td>
<td>F</td>
<td>48</td>
<td>Cutaneous lesion</td>
<td><em>C. ulcerans</em> (Elek−)</td>
<td></td>
<td></td>
<td><em>C. ulcerans</em> (Elek+)</td>
<td>Negative cat</td>
<td>Chronic renal failure</td>
</tr>
<tr>
<td>2006</td>
<td>M</td>
<td>73</td>
<td>Neurologic</td>
<td>Pseudomembranous pharyngitis</td>
<td></td>
<td></td>
<td>Date of last dose unknown</td>
<td>Return from Madagascar</td>
<td></td>
</tr>
<tr>
<td>2006</td>
<td>M</td>
<td>45</td>
<td>Cutaneous lesion</td>
<td><em>C. diphtheriae</em> (Elek+)</td>
<td>1986</td>
<td></td>
<td><em>C. ulcerans</em> (Elek+)</td>
<td>Cats and dogs not tested</td>
<td></td>
</tr>
<tr>
<td>2007</td>
<td>F</td>
<td>72</td>
<td>Otorrhea</td>
<td><em>C. ulcerans</em> (Elek−)</td>
<td>1970</td>
<td></td>
<td><em>C. auris</em> (Elek−)</td>
<td>Cats and dogs not tested</td>
<td></td>
</tr>
<tr>
<td>2008</td>
<td>M</td>
<td>58</td>
<td>Cutaneous lesion</td>
<td><em>C. ulcerans</em> (Elek−)</td>
<td></td>
<td></td>
<td><em>C. ulcerans</em> (Elek−)</td>
<td>No animal</td>
<td>Diabetic</td>
</tr>
<tr>
<td>2008</td>
<td>F</td>
<td>28</td>
<td>Cutaneous lesion</td>
<td><em>C. pseudotuberculosis</em> (Elek+)</td>
<td></td>
<td>2007</td>
<td><em>C. ulcerans</em> (Elek−)</td>
<td>Dog not tested</td>
<td>Diabetic</td>
</tr>
<tr>
<td>2008</td>
<td>F</td>
<td>71</td>
<td>Neurologic</td>
<td><em>C. diphtheriae</em> (Elek+)</td>
<td></td>
<td></td>
<td><em>C. ulcerans</em> (Elek−)</td>
<td>Return from Russia</td>
<td></td>
</tr>
<tr>
<td>2008</td>
<td>F</td>
<td>46</td>
<td>Cutaneous lesion</td>
<td><em>C. ulcerans</em> (Elek−)</td>
<td></td>
<td>2007</td>
<td><em>C. ulcerans</em> (Elek−)</td>
<td>Negative cats and dogs</td>
<td></td>
</tr>
<tr>
<td>2008</td>
<td>M</td>
<td>79</td>
<td>Cutaneous lesion</td>
<td><em>C. ulcerans</em> (Elek−)</td>
<td></td>
<td>2007</td>
<td><em>C. ulcerans</em> (Elek−)</td>
<td>Immunodeficient</td>
<td></td>
</tr>
</tbody>
</table>

*All the strains were PCR positive for the diphtheria toxin gene.*
opment will be undertaken after sequencing of the gene. All isolates were harbouring the tox gene in their genome but only 2 over 3 isolates of *C. pseudotuberculosis* and 7 over 12 isolates *C. ulcerans* were producing diphtheria toxin.

Throat swabbing of family members and social contacts was implemented for 12 patients: all four cases infected with *C. diphtheriae*, four cases with pseudomembranous pharyngitis due to *C. ulcerans*, and four cases with a leg ulceration. The number of contacts swabbed per case ranged from zero to over 100, with a median of five. All cultures remained negative. After testing, contacts received antibiotic prophylaxis. Information on immunization offered to contacts was available for 10 patients: the number of booster doses administered ranged from 2 to 60 with a median of five. No secondary cases occurred.

4. Discussion

No diphtheria cases were reported in France in the 1990s. Since 2002, four imported or imported-case-related cases due to *C. diphtheriae* have occurred, confirming the need for a high level of vaccination coverage in children and adults (particularly for travellers to endemic areas), and for sustained surveillance. In 2002, a survey was carried out in a representative sample of French households, through self-administered questionnaires for adults aged 16 and over, and found that only 33.7% of those surveyed had received a booster dose in the last 15 years, decreasing to 10.5% in those aged 64 and over [8]. At that time, 10-year-old diphtheria boosters were not included in the immunization schedule, and were mainly given to people travelling abroad or, via a combined diphtheria–tetanus–poliomyelitis vaccine, administered to comply with the recommendation of tetanus and poliomyelitis boosters every 10 years. In 2005, 10-year-old diphtheria boosters were also recommended, based on the recent availability of a new vaccine combining a low dose of diphtheria antitoxins with tetanus and poliomyelitis antigens, on diphtheria surveillance data, and on the most recent (1988) sero-epidemiological study which had found that almost 30% of the French population over 50 years old had no detectable antibodies for diphtheria or antibodies below the 0.01 IU/ml protection threshold [9]. Of 660 elderly people checked during the 2006 annual influenza vaccination at the Institut Pasteur in Paris, 28% had received a diphtheria booster in the previous 10 years. In the 60–69, 70–79 and 80+ age groups, 44%, 32% and 17% were up-to-date for diphtheria vaccination, respectively [10]. More time is needed to see the impact of the recent adult diphtheria booster recommendation on vaccination coverage. No study of nasopharyngeal carriage of corynebacteria has been undertaken in France.

Two of the four *C. diphtheriae* cases had classical respiratory diphtheria, and the diagnosis for the two others was made by systematic swabbing of the lesion. The latter received appropriate treatment, although diphtheria antitoxin was not available, and their contacts received appropriate management. The child case was only suffering from mild pharyngitis, probably because he had received four doses of vaccine. The adult case, infected in Madagascar, presented with cutaneous diphtheria, which is endemic in tropical countries. As described in the literature, this cutaneous form can lead to clusters with classical respiratory or cutaneous diphtheria [11]: rapid diagnosis is therefore crucial. Clinicians must collect swabs from patients returning from endemic areas who present with mild pharyngitis or skin ulcers, as was done for these two cases.

Diphtheria antitoxin is recommended for cases [12]. Three of the 19 cases developed toxigenic syndromes. Of these, two were infected with *C. ulcerans*, and developed neurological symptoms within a few days or weeks of admission. All cases in our series recovered, but on a recent review, four of the 28 published case reports of respiratory diphtheria-like illness caused by *C. ulcerans* died [13]. It is difficult to assess whether this outcome could have been avoided with rapid antitoxin treatment, especially since *C. diphtheriae* and *C. ulcerans* toxins may differ [14–16], which could lead to a less effective antitoxin therapy. However, as long as this hypothesis is not confirmed, cases infected with a toxigenic Corynebacterium strain should receive antitoxin. The occurrence of those toxin-producing diphtheria cases has recently led the Ministry of Health to stockpile several dozen diphtheria antitoxin doses.

Since the introduction of the new case definition in 2003, 12 cases due to *C. ulcerans* and 3 due to *C. pseudotuberculosis* have been reported. The distinction between *C. pseudotuberculosis* and *C. ulcerans* is sometimes difficult using bacteriological or ribotyping tests. The PCR targeting the pld gene [5] we introduced is perfectly reproducible and very useful for the identification of *C. pseudotuberculosis* isolates from animal origin. However, a validation is needed for isolates from human origin and an international reference isolate has to be chosen.

Not all isolates harbouring a tox gene in their genome are producing diphtheria toxin. This reinforces the need to test the toxin expression. One of the four Elek negative isolates was identified from a patient with respiratory obstruction: the membrane formation could be due rather to the bacteria than to the toxin. Another hypothesis is that during infection both expressing and non-expressing isolates were present and only the non-expressing one was collected. The isolates of the three patients presenting toxicogenic symptoms were Elek positive.

*C. pseudotuberculosis* is a rare zoonosis, and fewer than 30 cases have been described in the literature since the first reported case in 1966 [17]. Cases are most often linked to contact with goats. Apart from one eosinophilic pneumonia [18] and one eye infection [19], most human cases have had lymphadenitis [20,21]. The 22 isolates reported from Australia did not produce diphtheria toxin [20], and information is not available on the other reported cases. Since the detection of the first *C. pseudotuberculosis* isolate, the French diphtheria case definition has included any toxin-producing strain, in order to better describe the disease and possible complications linked to the toxin.

Cattle have classically been considered as the main reservoir of *C. ulcerans*, but recently infections have been described in cats and dogs [16,22]. Among the 12 *C. ulcerans* infections, none had contact with cattle, and 10 had contact with domestic animals: two of six animals tested were found to be carriers of *C. ulcerans* isolates harbouring the same ribotypes. This result is in favour of a domestic animal reservoir or novel ways of transmission: these hypotheses should be confirmed by the systematic sampling of animals that have been in contact with human cases. Unfortunately, in France, animal sampling must be paid for by the owner and is not performed systematically. One of the dogs treated had a positive control sampling. This could be due either to treatment failure or to a new contamination. As the prevalence of *C. ulcerans* carriage among domestic animals is unknown, control measures are unclear. In case of a high prevalence, it would be useless to investigate and treat animals, and better to reinforce human protection. If prevalence is low, investigation and treatment of the animal would make sense. Prevalence studies in domestic animals would help to answer this question.

Uncertainties are not related to treatment alone, as the efficacy of the vaccine against non-*C. diphtheriae* diseases is not known [23]. In our series, and according to the information we have, two of the non-*C. diphtheriae* cases had recently received a dose of vaccine, but did not develop severe disease. Up-to-date immunization should be encouraged.

Analysis of 50 of 59 *C. ulcerans* isolates received at the Streptococcus and Diphtheria Reference Unit (SDRU) in the United
Kingdom between 1986 and 2003 showed that of 45 toxin-producing strains, 30 were collected from women [22]; this seems to confirm the female predominance found in our small series. This reflects partly the predominance of women among the elderly, as 11 of the 15 cases were older than 50 years. Women have also been found to be less protected against diphtheria than men over 40 years [9]. They are probably also more exposed to domestic animals.

Human-to-human transmission of C. ulcerans has never been documented, although it was suspected in one instance [24]. However, because of our limited knowledge, the current recommendation includes the collection of throat swabs from close contacts, followed by antibioprophylaxis, as in infections due to C. diphtheriae [25]. In our series, no secondary cases occurred, and contact tracing and swabbing results are in favour of the absence of human-to-human transmission of C. ulcerans. Nevertheless, contact tracing was not systematically performed for cases suffering from skin ulcers or lymphadenitis, especially when the lesion had developed over a long period of time, preventing the identification of reasonable numbers of potentially exposed contacts.

Surveillance data may underestimate the reality of the epidemiology of toxin-producing corynebacteria in France, because in the absence of swabbing, some cases may not be identified. However, diphtheria is still a very rare disease which seems to be now mostly linked to contact with animals. Our surveillance data highlight the importance of high vaccination coverage in the population, and the need for enhanced surveillance. This would contribute to better understanding of the new epidemiology of diphtheria. In the meantime, a revision of the French diphtheria control measures guidelines, taking into account the available surveillance data, will be conducted in the near future.

We thank the Ddass, the clinicians and the bacteriologists who collected the data.

References