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Review

A review of the international issues surrounding the availability and demand for diphtheria antitoxin for therapeutic use

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ABSTRACT

Diphtheria treatment requires early administration of diphtheria antitoxin (DAT), an immunoglobulin preparation that neutralises circulating diphtheria toxin. Here, we review issues relating to the supply and use of DAT and assess its availability by means of an international survey. Results showed that several countries do not currently hold DAT stockpiles due to low prevalence, and hence perceived risk of diphtheria, and/or difficulties in obtaining DAT supplies. The potential for importation of cases into any country exists globally, since diphtheria remains endemic in many regions. It is therefore important that DAT be readily available – particularly since waning diphtheria immunity has been observed among adult populations in countries with good vaccination coverage. Options for diphtheria therapy are discussed.

Contents

1. Diphtheria

Diphtheria is an acute bacterial disease with a considerable case fatality rate caused by toxigenic strains of corynebacteria. Diphtheria toxin (DT) is the major virulence factor for these organisms, and contributes to the formation of a pseudomembrane in the nasopharynx of affected individuals. The colonising organisms are rarely found outside the local area of infection but the toxin is absorbed into the circulatory system where, when disseminated, it is able to cause systemic complications such as myocarditis and neuritis [1]. Three toxin-producing species have been identified; *Corynebacterium diphtheriae* is most commonly associated with communicable disease in humans, *Corynebacterium ulcerans* and *Corynebacterium pseudotuberculosis* are both less common in humans globally and are traditionally associated with contact with farm animals or dairy products. Recent cases of *C. ulcerans* have been associated with companion animals [2–4]. Toxigenic *C. diphtheriae* and *C. ulcerans* can cause both classic respiratory and systemic diphtheria, as well as other clinical presentations such as cutaneous diphtheria, which is more common in tropical areas of the world. Toxigenic *C. pseudotuberculosis* infections are usu-

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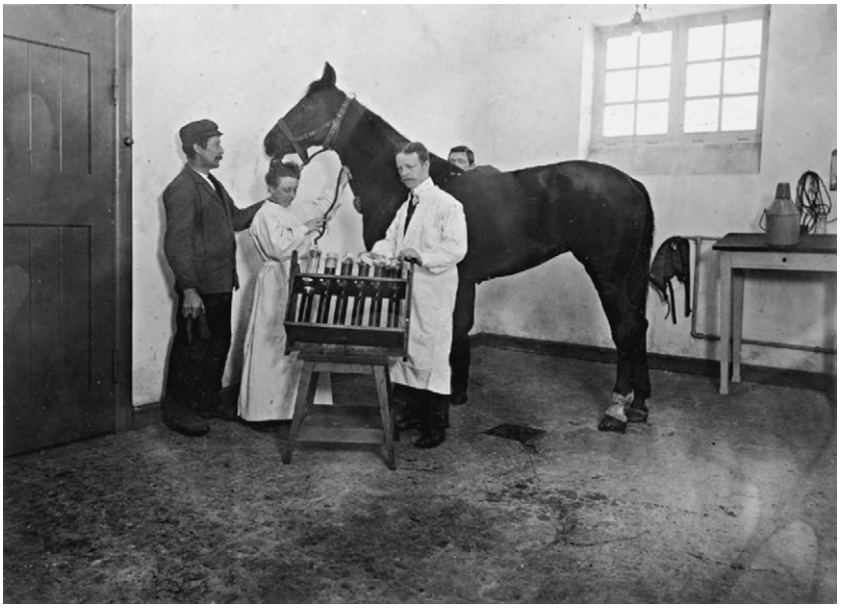

Fig. 1. Bleeding of a diphtheria horse, Statens Serum Institut, Copenhagen, 1904. With permission from Statens Serum Institut.

ally associated with lymphadenitis [5]. The DT gene is carried by a family of closely related bacteriophages (corynebacteriophages) that can integrate into the bacterial chromosome and convert non-toxigenic, non-virulent strains into toxigenic, highly virulent species [6,7]. However, transformation of a non-toxin-producing strain to a toxigenic organism is believed to occur rarely in nature.

2. Diphtheria toxin

Diphtheria toxin is synthesised and secreted as a single polypeptide, pro-enzyme that is cleaved and reduced *in vivo* to produce a toxic protein consisting of A and B fragments [8]. The B subunit contains the receptor binding and translocation domains of the toxin and the first step in the intoxication of eukaryotic cells by DT is the binding of toxin to specific cell surface receptors [9]. The receptor for DT was identified as the heparin-binding epidermal growth factor-like growth factor precursor (pro-HB-EGF) [10,11]. After binding of the toxin B subunit to the receptor, the toxin is internalised by receptor-mediated endocytosis. The low pH within the endosome causes a conformational change in the toxin molecule, facilitating translocation of the catalytically active A subunit of the toxin into the cytoplasm [12]. Once inside the cytoplasm, the A subunit, an ADP-ribosyltransferase, exerts its cytotoxic action by ADP-ribosylating elongation factor 2 (EF-2) thereby inhibiting cellular protein synthesis. The toxin has an estimated lethal dose for humans of $\leq$0.1 μg/kg [13]. The DTs of *C. diphtheriae* and *C. ulcerans* have been shown to be 95% identical; differences between these two DTs are mainly located in the translocation and receptor-binding domain of the B subunit. In contrast to *C. diphtheriae* DT, the DT of *C. ulcerans* seems to be much more heterogeneous [14].

3. Diphtheria therapy

Whilst diphtheria is an increasingly rare disease in the majority of developed countries, when cases do arise they can be severe and require a rapid and robust public health response. Case fatality rates worldwide remain high (>10%) [15]; a recently reported case fatality ratio (CFR) for Latvia for 2002–2007 was 9% [16]. Outside endemic areas CFRs can be even higher; delays in diagnosis and hence appropriate treatment have been reported [17]. The most effective treatment for diphtheria is early administration of diphtheria antitoxin (DAT), along with appropriate antimicrobial therapy to eliminate the corynebacteria from the site of infection thus stopping ongoing toxin-production. The protective effect of DAT has also been demonstrated *in vitro* and *in vivo* for *C. ulcerans* and is a treatment option for diphtheria caused by *C. ulcerans* [18]. However, in practice DAT is given based on clinical diagnosis, usually prior to laboratory confirmation [19]. DAT is a preparation of immunoglobulins or immunoglobulin $F(ab')_2$ fractions produced from immunisation of horses, that neutralises circulating DT. Emil von Behring won the first Nobel Prize for medicine in 1901 for his work on "Serum Therapy in Therapeutics and Medical Science" where he noted the importance of early use of diphtheria serum in order to achieve successful "detoxication of the bacillus poison" [20]. The antitoxin will only neutralise circulating toxin which has not bound to tissue; it is therefore critical that DAT is administered as soon as a presumptive diagnosis has been made without waiting for bacteriological confirmation [1]. A study of fifty patients with diphtheritic polyneuropathy in Riga, Latvia found antitoxin to be ineffective if administered after the second day of diphtheritic symptoms [21]. Aside from improved methods to refine or purify the equine serum, little has changed in diphtheria serotherapy since its introduction in the late 19th century and its continued use today, over 100 years later.

4. Diphtheria antitoxin supplies

Historical documents suggest that even in the pre-vaccine era the supply of DAT could be problematic, particularly in remote areas. 'The Serum Run of 1925' describes life-saving supplies of antitoxin being urgently 'mushed' across the snow by huskies in Alaska to reach a diphtheria epidemic in Nome [22]. Later, in an account of nursing during World War II, Barbara Brooks Tomblin describes problems with the supply of DAT and waiting 'as long as forty hours' for it to arrive [23].

In the early 1900s many countries (Denmark (Fig. 1), France [24], Germany [25], Canada [26], USA (Fig. 2) and UK [27] to name a few) produced their own therapeutic antitoxin preparation from horses. Fig. 1 shows the bleeding of a horse for production of diphtheria antitoxin at the Statens Serum Institut in Copenhagen, Denmark in 1904. Except for the director, the complete staff of the institute were present in the photograph. The description accompanying the