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ScienceDirect
Vaccine 25 (2007) 7636–7640
Vaccine
www.elsevier.com/locate/vaccine

Cattle immune response to botulinum type D toxoid: Results of a vaccination study

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Received 22 June 2007; received in revised form 20 August 2007; accepted 27 August 2007
Available online 17 September 2007

Abstract

Cattle botulism is a food-borne intoxication caused by the ingestion of preformed botulinum neurotoxins (BoNT) of serotypes B, C, or D. Protection in cattle against botulinum intoxication is based on the presence of specific serum neutralizing antibodies upon exposure. Outbreaks in vaccinated cattle have raised concerns about vaccine quality and efficacy. To this end, three different immunization protocols and the effect of maternal anti-BoNT/D antibodies, at the priming dose, were analyzed in 2-month-old dairy calves. Based on previously determined protective anti-BoNT/D antibody levels analyzed in field outbreaks, the immune response to type D toxoids was analyzed using an in-house ELISA system. Here we show that using the current vaccination strategy of using a priming dose in 2-month-old calves followed by booster doses after 4 weeks and annually thereafter, did not result in continuous protective levels of anti-BoNT/D antibodies. As a result of this vaccination protocol, only 15–31% of cattle in parities 1–3 were protected at the time of the annual booster. Vaccination study in calves indicated that adding a 6-month booster dose to the current protocol resulted in continuous protective levels of anti-BoNT/D antibodies well above the cut-off protective levels. The presence of maternally derived anti-BoNT/D antibodies did not interfere with the immune response to toxoids that can be administered to 2-month-old calves.

Keywords: Botulinum toxoid; Vaccination protocol; ELISA; Maternal immunity

1. Introduction

Botulism is a fatal disease manifested by muscular paralysis caused by the effect of neurotoxins produced by the bacteria *Clostridium botulinum, Clostridium baratii,* and *Clostridium butyricum*. *C. botulinum* produces all seven known neurotoxin serotypes (A–G), whereas *C. baratii* and *C. butyricum* produce only one serotype each (F and E, respectively) [1]. Botulinum neurotoxin (BoNT), the most lethal substance known, affects all mammals, birds, and fish. Worldwide, field outbreaks of cattle botulism were caused by serotypes B, C, and D, whereas in Israel the most prevalent serotype is D [2].

In recent years, sporadic cases and massive outbreaks of cattle botulism occurred in Europe, North America, South America, Australia, and Israel [3–14]. Most notable is the marked increase in reported incidents of suspected botulism in cattle in the UK since 2003 [15] and in Israel since 2002 [2]. However, the current situation in these two countries is very different. For example, in Israel all livestock have been routinely vaccinated since the late 1970s after massive outbreaks, resulting from introducing unprocessed chicken manure as a feed additive [16]. For two decades, botulism vaccination with various bivalent type C and D toxoids was considered highly successful in Israel. However, in June 2002, a large type D botulism outbreak occurred in southern Israel, involving 28 dairy farms, killing more than 600 dairy cattle [17]. All animals were routinely vaccinated with a bivalent toxoid, using the currently recommended vaccination protocol: with a priming dose at the age of 2 months, followed

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doi:10.1016/j.vaccine.2007.08.051

4 weeks later and annually by booster doses. In this outbreak, botulism occurred 10–12 months after the last annual booster and affected mainly calves and heifers. This unexpected outbreak in vaccinated animals is in contrast with previously reported data from Australia [18] and South Africa [19] where similar botulinum toxoids (albeit with different protocols) were used raised concerns about the cattle vaccine's quality, efficacy and protocols in use. Cattle botulism outbreaks in Israel are characterized by rapid onset and high morbidity and mortality [2]. Therefore, protection is entirely dependent on the presence of specific serum neutralizing antibodies upon ingestion of the preformed toxin. We have previously used field outbreaks to determine the protective levels of anti-BoNT/D antibodies following vaccination with the commercially available bivalent C and D toxoids. Here we report the analysis of anti-BoNT/D antibody levels following routine vaccination of cows on a commercial dairy farm using the predetermined cut-off level as a measure of vaccination efficacy. Furthermore, the result of our previous [2] and this initial study casted doubts on our current vaccination protocols. To this end, three different immunization protocols and the effect of maternal anti-BoNT/D antibodies at priming dose were analyzed in 2-month-old dairy calves.

2. Materials and methods

2.1. Animals

All animals participating in this study were part of a single Israeli Holstein commercial dairy herd consisting of 600 milking cows. Cows were housed in loose housing systems in large, completely covered open sheds, fed total mixed ration (TMR) ad lib and were milked three times per day, with an average annual milk production of 12,000 kg per cow. TMR included wheat or corn silage, concentrates and a mineral and vitamins premix. All cows and calves were identified by ear tags and/or freeze markings. Computerized dairy herd management systems were used for electronic cow identification as well as storage of all demographic data and individual vaccination records (Afimilk^{TM} and NOA, Israel Cattle Breeders Association). During the preceding 5 years no cases of botulism were diagnosed or suspected in the study farm or neighboring farms.

All animals in the herd were vaccinated by subcutaneous injection of a priming dose at the age of 2 months and received booster doses 4 weeks later and once a year thereafter, with one of the commercially available brands of type C and D bivalent toxoids (CSL Limited, Parkville, Victoria, Australia (2.5 ml per dose); Prondil S.A., Montevideo, Uruguay (2 ml per dose)). In previous experimental studies of cattle and mice, no difference could be discerned in the immune response to these two toxoids (Steinman A. and Shpigel N.Y., unpublished results [2]).

2.2. Analysis of anti-BoNT/D antibody levels in vaccinated cattle

Sera samples were collected from 129 Holstein-Friesian cows of various parities (defined as the number of calvings a cow has delivered) and replacement heifers (animals before first calving at about the age of 24 months). Sera samples were collected 1 year after the last annual booster vaccination before the annual booster was administered. Serum was separated by centrifugation ($2000 \times g$) and kept at $-80\,^{\circ}C$ until analyzed. The levels of specific anti-BoNT type D antibodies in the sera were determined using an in-house ELISA, as described previously [2].

2.3. Vaccination study

Thirty-two 2-month-old female Holstein-Friesian calves were subcutaneously immunized with a priming dose, and by use of their brand numbers they were randomly divided into three experimental groups of booster dose schedules. Group 1 calves were immunized at 4 weeks; group 2 at 4 and 12 weeks; and group 3 at 2, 12, and 25 weeks after the initial priming dose (Fig. 1). All calves were immunized again 53 weeks after the initial injection (annual booster). Blood samples were collected from all calves before the first injection and after 2, 4, 12, 17, 25, 31, 43, 53, and 55 weeks.

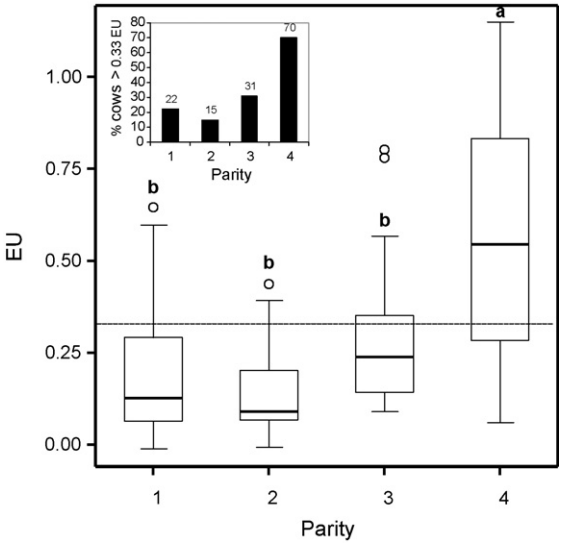

Fig. 1. Anti-BoNT/D antibody levels in cows that underwent calfhood vaccination (at 2 and 3 months of age) and annual boosters with bivalent (C and D) botulinum toxoid. Sera samples were obtained from each cow just before the annual booster and assayed individually for the presence of IgG antibodies against BoNT/D. Antibody levels in ELISA units (EU) are displayed by Box-plot diagrams. The botulism protective titer threshold (0.33 ELISA units) is denoted by a horizontal dashed line. The inset diagram displays the proportion (%) of cows in each parity group with anti-BoNT/D serum antibodies above the protective threshold. The means of antibody levels were compared among parity groups by analysis of variance; parity groups with different superscripts differed significantly when tested by one-way analysis of variance – Bonferroni comparison of means, $P < 0.05$.