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Dose-response modelling of staphylococcal enterotoxins using outbreak data

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Abstract

Staphylococcal food poisoning (SFP) is one of the most common food-borne diseases and results from the ingestion of staphylococcal enterotoxins (SEs). Yet, small amount of data are available for establishing a dose response. The objective of this work was to build a dose response relation based on the systematic investigations carried out during recent years in France. Over the period 2010-2014, more than 60 SFP outbreaks involving SEs, mainly from France, were microbiologically investigated. The enterotoxins were characterized as well as quantified. Attack rates, appearance times and natures of symptoms collected during epidemiological investigations were related to microbiological data. The outbreaks collected focused on enterotoxins SEA, SEB, SEC, and SED. Distribution of appearance times of symptoms and their natures were not influenced by the type of enterotoxins. The US EPA benchmark dose (BMD) methodology was then used to establish dose response. Attack rates of SFP outbreaks were modelled as a function of ingested doses and a BMD have been estimated for SEA.

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Keywords: benchmark dose; dichotomous data; uncertainty

1. Introduction

Staphylococcal food poisoning (SFP) is one of the most common food-borne diseases and results from the ingestion of staphylococcal enterotoxins (SEs). SEs are highly heat resistant, preformed, large peptides produced by some *S.*

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aureus strains. According to the official European Union data from 2013[1], 386 foodborne outbreaks were attributed to *Staphylococcus* spp. in 12 Member States, representing 7.4% of all reported foodborne outbreaks.

Doses of approximately 20 to 100 ng have been reported effective in causing SFP[2]. However, these concentrations are taken from a limited number of food outbreaks. Because SE concentrations are rarely measured, there here is small amount of data available for dose response. The objective of this work was to establish a dose response based on the systematic investigations carried out during recent years in France.

2. Materials and Methods

2.1. Enterotoxin identification and quantification

Food involved in SFP outbreaks were tested for the presence of enterotoxins (A to E) according to the European Screening Method of the European Union Reference Laboratory (EURL) for coagulase positive Staphylococci (CPS), consisting of an extraction followed by a dialysis concentration step coupled to detection with the Vidas SET2 kit, a combined qualitative detection test (BioMerieux, Marcy l'Etoile, France). SE-positive samples were further analyzed by quantitative double sandwich ELISA, the confirmatory method of the EURL for CPS[3] to quantify the amount of each enterotoxin produced.

2.2. Nature of effects

The distribution of reported symptoms for the selected SFP was represented with Venn diagrams. The diagrams were constructed using the venneuler R package. The distribution of mean incubation periods (i.e. the onset of symtoms after consuming contaminated food) observed in the 63 outbreaks as well as the distribution of individual incubation periods within an outbreak were characterized using R 3.1 software and the "fitdistrplus" package[4].

2.3. Dose response modelling

The benchmark dose (BMD) approach was used. It involves dose-response modeling to obtain BMDs, i.e. dose levels corresponding to specific response levels. For this, we selected (i) the outbreaks on which to base BMD calculations, (ii) the benchmark response value, (iii) tested the models to use in computing the BMD, (iv) assessed the models fit and carried out and model comparison and (v) computed the confidence limit for the BMD (i.e., the BMDL). The US EPA Benchmark Dose Software[5] was used.

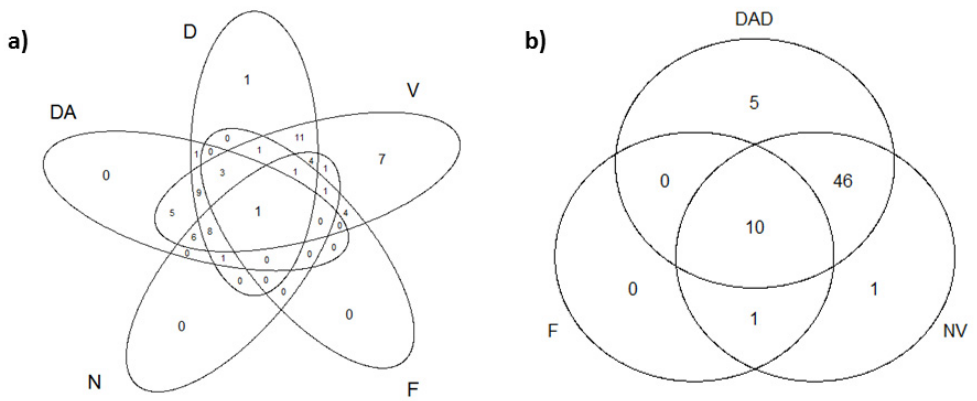

Fig. 1. Repartition of the identified symptoms in the 63 SFP outbreaks. Venn diagram with (a) individual symptoms: diarrhea (D), abdominal pain (DA), vomiting (V), nausea (N) and fever (F); and (b) grouped symptoms: abdominal pain and diarrhea (DAD), nausea and vomiting (NV) .

3. Results and discussion

3.1. Characteristics of SFP outbreaks

Data of 63 SPF outbreaks were collected. The study focused on enterotoxins SEA, SEB, SEC, and SED. These SEs were present alone or in combination in the suspected foods. SEA was the most detected enterotoxin. This toxin was detected alone in 36 outbreaks and associated with other toxins in 18 outbreaks. SEC and SEB were detected as the unique involved enterotoxins both in three cases.

Five symptoms are usually reported during foodborne outbreak investigations (diarrhea, abdominal pain, vomiting, nausea, and fever). The five symptoms were found at least one in SFPs. Vomiting is the most common symptom. Fig.1 shows the repartition of the five symptoms taken individually or grouped by similarity. The presence of more than two symptoms in outbreaks could not be related to the presence of several SEs in foods.

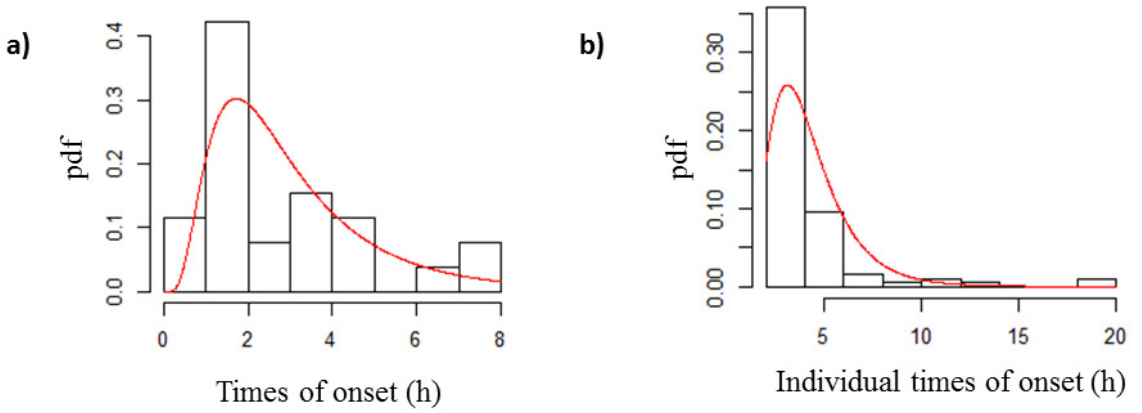

Fig. 2. (a) Distribution of times of onset of symptoms of the 63 SFP outbreaks; (b) Person to person variability of times of onset of symptoms for one outbreak.

The log-normal distribution was fitted to the observed distribution of times of onset of symptoms. According to outbreaks, symptoms can appear in less than one hour after ingestion of the contaminated food up to eight hours (Fig. 2a). This distribution was not influenced by neither the presence of several SEs nor the presence or large quantity of SEs. For three outbreaks, the individual times of onset of symptoms were available. Fig. 2b shows that within one of these outbreaks, they ranged from less than one hour up to 20 hours.

3.2. A BMD for SEA

The attack rates, i.e. the number of persons with symptoms divided the number of exposed people, according to the doses of SEA ingested were used for dose response modelling and Benchmark Dose (BMD) establishment. BMD may then be defined as the dose that induces effects in 10% of the exposed population (BMD_{10}). Usually the BMD_{10} is not the value of reference. The lower 95%-confidence interval of the BMD_{10}, also called the Benchmark Dose lower limit ($BMDL_{10}$) is preferentially used.

SEA was the only enterotoxin for which sufficient data were available for dose response modelling. All the outbreaks where other SEs were identified and/or quantified along with SEA were not included. Within the 36 outbreaks with SEA alone, only 14 outbreaks were used for dose response. For the remaining 22, the attack rates were not known. Generally for these excluded outbreaks, the number of exposed people was unknown or highly uncertain. Fig. 3 shows the fitting of Weibull model. The $BMDL_{10}$ was estimated at 6.1 ng of SEA.

The estimate of $BMDL_{10}$ takes into account the uncertainty linked to attack rate. Yet, the assessment could be further

improved by taking into account the uncertainty on the ingested doses. The quantification of SEs in the years to come will permit to adopt the same approach for SEB, SEC, and SED. The outbreaks involving several toxins will permit to check for potential synergy between SEs.

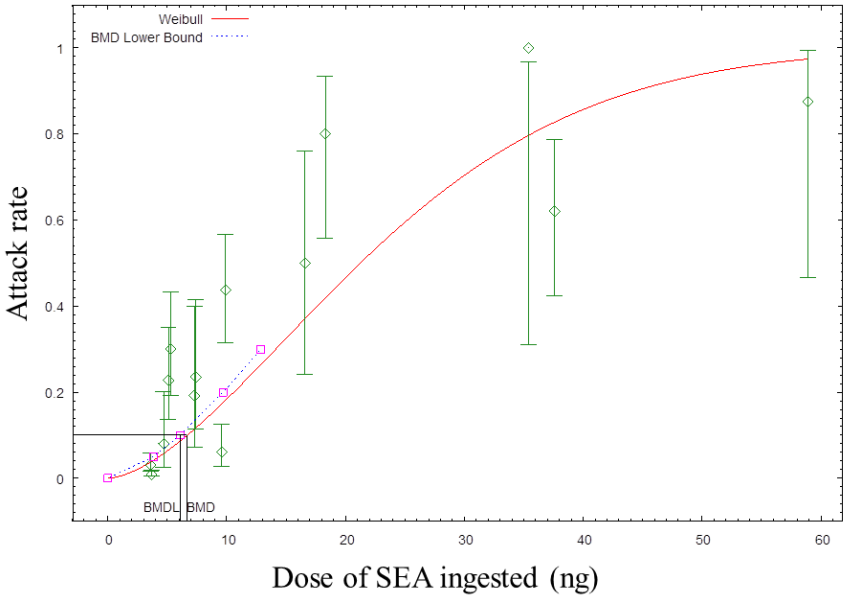

Fig. 3 BMD estimated from 14 SEA outbreaks. The curve is calculated using the Weibull model. The BMD10 is the dose that gives effect in 10% of the tested population. The BMDL10 is the dose corresponding to the lower 95%-confidence interval.

4. Conclusion

This model may be useful for risk assessment if there were a quantitative relationship between the number of CPS and enterotoxin production. More pragmatically, the estimated value of $BMDL_{10}$ provides a basis for determining the detection limit that should reach enterotoxin detection methods. For example, according to the available data under the assumption of a 100 g serving size, the limit of detection for qualitative methods should be lower than 0.06 ng/g for SEA.

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