Veterinary Microbiology 177 (2015) 398–402

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Short Communication

Occurrence of *Clostridium botulinum* neurotoxin in chronic disease of dairy cows

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ARTICLE INFO

Article history:
Received 16 January 2015
Received in revised form 11 March 2015
Accepted 12 March 2015

Keywords:
Clostridium botulinum
Botulism
Dairy herds
Chronic disease

ABSTRACT

Botulism caused by neurotoxins of *Clostridium (C.) botulinum* is a rare, but serious life-threatening disease in humans and animals. Botulism in livestock is usually caused by the oral uptake of *C. botulinum* neurotoxins (BoNT) via contaminated feed and is characterized by flaccid paralysis. In the recent past a new syndrome caused by BoNT in dairy cattle was postulated. It was supposed that *C. botulinum* is able to colonize the lower intestine and may subsequently produce neurotoxin. The continuous resorption of small amounts of these BoNT may then provoke the so called syndrome of "chronic" or "visceral" botulism involving unspecific clinical symptoms, reduced performance of dairy cows and massive animal losses in the affected herd. To test this hypothesis a case-control study was conducted involving 92 affected farms and 47 control farms located in Northern Germany. Fecal samples of 1388 animals were investigated for the presence of BoNT to verify the key requirement of the hypothesis of chronic botulism. BoNT was not detected in any of the fecal samples using the most sensitive standard method for BoNT detection, the mouse bioassay. Therefore, the existence of "chronic" or "visceral" botulism could not be proven.

1. Introduction

Botulism is caused by the bacterium *Clostridium* (*C.*) *botulinum* and is usually an intoxication in humans and also in animals, when the *C. botulinum* neurotoxins (BoNT) are ingested with contaminated food or feedstuff, respectively (Hatheway, 1990). So far, seven serologically differentiable toxin types (A–G) are known, a new serotype H was proposed recently which remains to be verified (Rossetto et al., 2014). Serotypes A, B, E and F are causing human and animal botulism, whereas types C and D are reported to cause predominantly animal botulism (Lindstrom and Korkeala, 2006; Lindstrom et al., 2010). Furthermore, BoNT serotypes are divided into subtypes based on their nucleic acid and amino acid sequence heterogeneity (Hill and Smith, 2013). Remarkable morphological and physiological differences are seen between

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http://dx.doi.org/10.1016/j.vetmic.2015.03.012

C. botulinum strains isolated from different sources. Four physiologically different BoNT producing groups of *C. botulinum* (I–IV) are described (Hatheway, 1990; Rossetto et al., 2014). Modern comparative sequence analysis also confirms that there are significant genetic differences between these groups (Rossetto et al., 2014). These findings also point to the fact that at present several different *Clostridium* species, the common characteristic of which is the production of orally effective neurotoxins, are subsumed under the name *C. botulinum*. Additionally, some strains of *C. baratii* and *C. butyricum* produce BoNT of the serotypes F and E, respectively (Rossetto et al., 2014).

BoNT are highly potent poisons and are considered to be the most toxic substances produced by living organisms (Gill, 1982). The seven known serotypes cause muscular paralyses by blocking the release of acetylcholine at the neuromuscular synapses. Due to the complexity of their toxic effect, the detection of *C. botulinum* neurotoxins represents a considerable diagnostic challenge (Dorner et al., 2013; Lindstrom and Korkeala, 2006). So far, BoNT are the only known bacterial toxins which are protected against environmental influences by non-toxic accompanying proteins. Based on current knowledge, these accompanying proteins are important for maintaining the toxicity of BoNT in the environment and during gastrointestinal passage. They also play an important role in passing epithelial barriers (Benefield et al., 2013; Gu et al., 2012; Lee et al., 2013, 2014).

Establishing the diagnosis bovine botulism is difficult and mainly based on the clinical picture and the exclusion of differential diagnoses (Stöber, 2002). As small amounts of BoNT are sufficient to cause severe disease, it is often difficult or impossible to detect the toxin in food or feed products, especially since it is not homogenously distributed throughout the matrix. Toxin detection in serum, organs or intestinal contents is also not always successful (CDC, 1998; Hatheway, 1990). As the pathogen occurs ubiquitously (Bell and Kyriakides, 2000; Espelund and Klaveness, 2014), detection of *C. botulinum* in the environment, in organs or in the intestinal contents is no proof of botulism. Therefore, the detection of BoNT is a prerequisite for the laboratory based diagnosis of botulism. In the recent years a number of detection methods for BoNT had been developed, one of these, the Endopep-MS, is demonstrating extraordinary high sensitivity for BoNT of several types in food (Kalb et al., 2015), but none has fully replaced the mouse bioassay (Dorner et al., 2013; Singh et al., 2013). The mouse bioassay still is the most sensitive and reliable detection method for biologically active BoNT in clinical samples. It is considered to be the standard method for botulinum toxin detection worldwide (Dorner et al., 2013; Lindstrom and Korkeala, 2006; Singh et al., 2013).

Human foodborne botulism is well known, but other forms of botulism are also recognized (Rossetto et al., 2014). Botulism can present as intestinal toxemia e.g. in infant botulism, when *C. botulinum* colonizes the intestine and then releases neurotoxin, which is absorbed into the bloodstream. As a result, infants suffer from descending motor weakness and flaccid paralysis (Midura and Arnon, 1976; Pickett et al., 1976). The insufficiently developed intestinal flora of infants up to one year may favor colonization with *C. botulinum*. Spores of *C. botulinum* are possibly ingested with honey (Hatheway, 1990). Infection of wounds with *C. botulinum* and subsequent botulism is known as wound botulism mainly in persons who inject drugs (Lindstrom and Korkeala, 2006; Rossetto et al., 2014).

A relatively common form, BoNT type C botulism, is seen in aquatic birds, particularly wild birds (Songer, 1997), but it is sometimes also seen especially in the summer season in domestic poultry (Songer, 1997). The bacterium *C. botulinum* is widespread in the environment, but botulism in livestock especially in cattle occurs only sporadic (Lindstrom et al., 2010). Botulism in cattle is mainly caused by BoNT types C and D. An association with poultry feces or litter has been observed (Livesey et al., 2004; Payne et al., 2011). BoNT types A and B were only rarely diagnosed in botulism cases of cattle (Lindstrom et al., 2010). In general, cattle intoxication is often caused by feed products which had been contaminated with carcasses, e.g. silages (Songer, 1997).

In addition to foodborne botulism in domestic animals, another form of disease in cattle has been discussed controversially. An untypical form of botulism is supposed to be caused by the colonization of the lower intestine with *C. botulinum* bacteria, subsequent production of BoNT and continuous resorption of small amounts of toxins resulting in chronic wasting of the affected dairy herd involving a complex of unspecific clinical symptoms, reduced performance and massive animal losses (Bohnel et al., 2001). The presumed trigger is a microbial imbalance in the digestive tract favoring the multiplication and toxin production of *C. botulinum*. It is assumed that this syndrome is multifactorial. This hypothesis has been proposed as "visceral" form of botulism (Bohnel et al., 2001). Especially, a significant number of dairy herds affected by chronic wasting condition in Northern Germany were suspected to suffer from "visceral" botulism (Bohnel and Gessler, 2012). Due to the unspecific clinical picture, a reasonable case definition based on clinical findings is still pending.

"Visceral" botulism was also discussed to be a sporadic, but severe zoonosis, when farmers or members of their families developed illness involving neurological syndromes (Dressler and Saberi, 2009).

The overall aim of this collaborative study was to investigate the cause of chronic disease in dairy cattle herds, which is associated with gradual wasting and can affect entire holdings. The crucial point of the hypothesis of "visceral" botulism is the production of BoNT by viable *C. botulinum* bacteria in the intestine of affected animals. The presence of BoNT in feces at detectable amounts is the key requirement of the hypothesis of chronic botulism. In fact, Bohnel et al. based their hypothesis on detection of BoNT in feces of affected animals using the mouse bioassay (Bohnel et al., 2001). Therefore, the confirmation of the presence of BoNT in the feces of affected animals applying the most sensitive analytical method for botulinum toxin detection i.e. the mouse bioassay can add more certainty to the hypothesis of "visceral" botulism in dairy cows.