



Epidemiological and epizootological aspects of botulism in Ukraine

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Botulism remains one of the most dangerous toxic infections in humans and animals, characterized by severe neuro-paralytic manifestations and a high mortality rate. The causative agent *Clostridium botulinum* is a naturally occurring anaerobe capable of long-term preservation in spore form and production of an extremely potent botulinum neurotoxin (BoNT). Despite considerable attention being paid to this disease and changes in European veterinary legislation, botulism remains a pressing problem worldwide and, in particular, in Ukraine, both in terms of veterinary safety and public health. The study was conducted to analyze the epizootological and epidemiological characteristics of botulism in Ukraine over the past 15 years among animals and 10 years – among humans. The data sources were reports from regional laboratories of the State Service of Ukraine for Food Safety and Consumer Protection, research results from the State Scientific and Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise, and information from the Public Health Center of the Ministry of Health of Ukraine. All cases were confirmed by laboratory tests (immunofluorescence assay, bioassay on laboratory animals). For statistical analysis, binomial confidence intervals (BCI) calculated using the Klopfer-Pearson method were used, and for visualization, the QGIS 3.16 program was used with cartographic layers of the administrative boundaries of Ukraine. During the period under review, 7,031 positive cases of botulism were registered among animals and in feed, of which 77.9% (BCI, 77.0–78.9%) were isolates from feed and 22.1% (BCI, 21.1–23.1%) were from animals. Among the affected farm animals, pigs (41.6%), cattle (21.9%), and horses (7.8%) dominated. A significant number of cases were registered among cats, dogs, minks, foxes, wild rodents, and poultry, which were grouped into the category “others” and accounted for 28.8% (BCI, 26.5–31.1%). The highest number of positive cases among animals and feed was recorded in the Luhansk (80.2%), Vinnytsia (12.7%), and Donetsk (5.0%) regions. At the same time, between 2018 and 2024, the number of tests decreased significantly: only one positive sample was found in feed, which is more than 500 times less than in the previous seven years. Between 2015 and 2024, 1,070 cases of botulism were registered in humans, mainly in the central and northwestern regions. The results indicate a decrease in the intensity of diagnostic studies, which may lead to an underestimation of the real epizootic situation. It is necessary to restore systematic monitoring, feed control, and strengthen biosafety on farms as key elements of botulism prevention in Ukraine.

Keywords: *Clostridium botulinum*; epizootology; humans; farm animals; feeds; monitoring; mapping.

Introduction

The causative agent of the highly dangerous disease of botulism is *Clostridium botulinum* (its toxins). It is a gram-positive, anaerobic, spore-forming rod that causes a severe neuroparalytic disease registered in humans and vertebrate animals (Sobel, 2005). The disease is caused by botulinum neurotoxin (BoNT), which is a fairly potent biological agent (Rawson et al., 2023). BoNT metalloproteinase specifically cleaves soluble N-ethylmaleimide-sensitive attachment protein (SNARE) proteins in postsynaptic nerve endings, thereby preventing the release of neurotransmitters and blocking neural transmission to effector muscles (Rossetto et al., 2021).

The pathogen is virtually ubiquitous, and humans and animals regularly encounter the spore form of this bacterium (Rawson et al., 2023). *Clostridium botulinum* spores are often not a threat to animals and humans unless they germinate into vegetative cells that produce neurotoxin. In general, the digestive system of animals and humans is not a place that promotes spore germination, and they can often pass through the body without causing any harm, but if there is intestinal dysbiosis, the situation can become more complicated (Peck, 2009).

BoNT is divided into seven serotypes of toxins from A to G, with subtype variants designated within each toxin type with a sequential number in order of discovery (Peck et al., 2017). In 2016, whole-genome analysis and bioinformatic approaches revealed a new serotype

in the existing *C. botulinum* strain, strain 111, which also produced the BoNT/B2 neurotoxin. This serotype was named BoNT/X, and although the toxin has been shown to cleave a number of SNARE targets, it is unknown whether the BoNT/X gene cluster is expressed naturally (Zhang et al., 2017). This toxin, BoNT/FA, was briefly considered a new BoNT/H serotype until later analysis revealed it to be a chimeric toxin (Kalb et al., 2015; Maslanka et al., 2016). Toxin serotypes are currently classified based on the neutralization of toxins by monoclonal antibodies, while subtypes are mostly classified based on differences in amino acid sequences, although subtypes can differ by 2.6–31.6% at the amino acid sequence level (Smith et al., 2005; Hill & Smith, 2013). According to this criterion, only four serotypes have designated subserotypes: BoNT/A, BoNT/B, BoNT/E, and BoNT/F.

The use of biological agents for bioterrorism is a cause for global concern (Janik et al., 2019). BoNT is classified as one of the highest risk threats and is listed as a Category A agent by the US Centers for Disease Control and Prevention (CDC). Due to the possibility of using the toxin as a biological weapon, the storage of neurotoxic BoNT strains requires enhanced security measures. BoNT is a rather dangerous biological agent with the lowest lethal dose among all known natural substances (Rawson et al., 2023). The lethal dose for humans (LD₅₀) depends on the route of entry into the body; for example, for a body weight of 70 kg, the lethal doses are 70 µg when ingested, 0.7–0.9 µg when inhaled, and 0.09–0.15 µg when concentrated

BoNT/A toxin is administered intravenously (Amon et al., 2001). Botulism is reported on all continents, with varying incidence rates in different years. In all species, the disease manifests as flaccid paralysis, including respiratory and cardiac failure (Le Bouquin et al., 2022). This disease affects many animal species, mainly birds and cattle (Rasetti-Escargueil et al., 2019), fur animals (minks, foxes, etc.) in Northern European countries (Lindström et al., 2004; Le Bouquin et al., 2022) and horses in the United States (Galey, 2001). Intoxication leads to corresponding clinical signs in cattle. The entry of preformed toxins into the body through food, water, or any contaminated substance causes clinical signs of botulism. Botulism in birds is the result of ingesting *C. botulinum* spores. Spores that enter the body germinate, multiply, and produce toxins mainly in the cecum. Absorption of the toxin formed in the digestive tract causes corresponding symptoms (Popoff, 1989). Botulism in humans is not frequently reported. Several types of botulism are recorded in humans, depending on the mode of infection and exposure to the toxin (foodborne, intestinal, wound, iatrogenic, inhalation) (Anniballi et al., 2014). Foodborne botulism and infantile intestinal botulism are the two most common forms reported worldwide (Rasetti-Escargueil et al., 2020).

Foodborne botulism occurs after consuming food contaminated with preformed botulinum-like toxin (BoNT), and even a small amount (a few milligrams) of contaminated food is enough to cause symptoms and possibly death if medical attention is not provided (Peck, 2009). Symptoms usually appear 12–72 hours after consumption of contaminated food and also depend on the amount of contaminated food consumed (Amon et al., 2001). The study of foodborne botulism has historically been associated with significant human morbidity from this form and has been associated with unprocessed or undercooked foods, both at home and in commercial food production facilities. When using preservation methods such as fermentation and marinating, and subsequently preserving or bottling products without prior heat treatment, such products pose a significant threat because they create the anaerobic conditions necessary for the germination of clostridial spores and the growth of vegetative forms of bacteria (Sobel, 2005).

Foodborne botulism is also a problem for domestic and wild animals, as outbreaks of this disease among farm animals and waterfowl often spread rapidly and lead to devastating epidemics through corresponding transmission cycles in the environment (Rawson et al., 2023). The growth of *C. botulinum* bacteria in anaerobic conditions (often decomposing organic matter) leads to the accumulation of significant concentrations of BoNT, which can enter the bodies of animals, birds, or fish in various ways, often leading to death. Death usually occurs from the direct action of the neurotoxin (lethal dose), as well as after consuming a non-lethal amount, which causes the animal to become depressed, after which it becomes prey to predators or is displaced by healthy animals in competition for resources. Such contaminated carcasses, if consumed by predators, will also cause disease in the latter (Espelund & Klaveness, 2014). This cycle of infection can be exacerbated if the neurotoxin enters the body of insects that are insensitive to BoNT and other invertebrates (larvae, worms, mollusks, etc.) and is subsequently consumed by animals that are quite sensitive to the toxin (Duncan & Jensen, 1976). Outbreaks of the disease in animals are mostly associated with BoNT C and D toxin serotypes, while botulism in horses is mainly caused by BoNT B (Johnson et al., 2016).

Different animal species show different sensitivities to different BoNT toxin types. Natural botulism in animals is mainly caused by BoNT C, D, and mosaic variants CD and DC, with the first being more common in birds and the second – in cattle, while human botulism is mainly caused by types A, B, and E, and to a lesser extent by type F. Botulism is not a contagious disease, as there is no direct transmission from sick animals or humans to healthy individuals. Botulism is transmitted through the environment, in particular through food contaminated with *C. botulinum* spores stored in conditions conducive to the growth of this pathogen and the production of toxins. The high prevalence of botulism types C, D, and variants DC and CD in farm and wild birds, and to a lesser extent in cattle, increases the risk of transmission to humans. However, human botulism is much less common than animal botulism, and types C and D botulism in humans are rare. Healthy animals that are carriers of *C. botulinum*

group II, such as *C. botulinum* type E in fish in the northern hemisphere and *C. botulinum* B4 in pigs, pose a more widespread risk of transmitting botulism to humans. Less common types of botulism in animals, but which still pose a risk of transmission to humans, occur sporadically, for example, cases of type E botulism in farm chickens in France (1998–2002), botulism type B in cattle in the Netherlands (1977–1979), botulism types A and B in horses, or botulism type A in dairy cows (Egypt, 1976) (Rasetti-Escargueil et al., 2019).

Despite the measures taken to control and prevent the spread of botulism in Ukraine, it has not yet been possible to prevent the incidence of the disease in humans and animals in certain regions of our country. Many aspects of botulism in Ukraine have not been thoroughly studied. A preliminary analysis of the epizootic situation with this disease in Ukraine shows that the interest of veterinary specialists in this problem has significantly decreased since 2017. Our work characterizes the epizootic and epidemic situation with botulism over the past 10–15 years using cartographic visualization.

Materials and methods

The authors of the article conducted a retrospective epizootic and epidemic analysis of the incidence of botulism in animals (for the period 2010–2024) and humans (2015–2024) in Ukraine. The study characterized the epizootic situation regarding botulism among various animal species (farm animals and the “others” category, which included dogs, cats, foxes, minks, birds, wild rodents, etc.).

The source of data for the analysis of botulism cases in Ukraine among various animal species was reports from regional laboratories of the State Service of Ukraine on Food Safety and Consumer Protection (SSUFSCP) and data from studies by the State Scientific and Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise (SSRILDVSE) (Kyiv, Ukraine). The data from forms 1-Vet and 2-Vet for 2010–2024 were systematized and analyzed. All cases of botulism cited in the publication were confirmed by diagnostic tests performed in accredited laboratories (immunofluorescence assay, bioassay on laboratory animals). Data from the Public Health Center of the Ministry of Health of Ukraine for the period 2015–2024 were used to analyze the epidemiological situation with botulism. Due to military actions, the sample did not include data from the temporarily occupied territories of the Autonomous Republic of Crimea, the city of Sevastopol, and parts of Donetsk and Luhansk regions.

Statistical data were performed by the binomial confidence intervals (BCI) that were calculated to assess a seroprevalence using the Clopper-Pearson exact method with a confidence level of 0.95 using the R epitools software package (Ausvet, version 2020, Australia) (<https://epitools.ausvet.com.au>). Mapping of data on the number of botulism cases among people and different animal species, including positive samples of pathogen isolation in feed by Ukrainian regions, was performed using the freely available Quantum GIS software version 3.16.0 (International Quantum GIS Project, 2020, Germany). (www.qgis.org/ru/site/forusers/download.html). Vector layers representing the borders of Ukrainian regions were downloaded from www.diva-gis.org/Data. A quantile with 6 data classes was selected for classification.

Results

Systematized research results show that during the period 2010–2024, the causative agent of botulism (or its toxin) was isolated from various animal species and animal feed in 7,031 cases. The highest number of positive cases was registered in the Luhansk – 5,642 (80.2% of the total number; BCI, 79.3–81.2%), Vinnytsia – 895 (BCI, 12.0–13.5%) and Donetsk – 349 (BCI, 4.5–5.5%) regions. A smaller number of cases were detected in Odesa – 47 (BCI, 0.5–0.9%), Zaporizhzhia – 39 (BCI, 0.4–0.8%), and Ivano-Frankivsk – 31 (BCI, 0.3–0.6%) regions. In Lviv, Zhytomyr, Ternopil, Khmelnytskyi, Sumy, and Chernihiv regions, a total of 28 cases of botulism (BCI, 0.2–0.6%) were registered during the period under review. In general, positive cases of botulism among animals and the isolation of the pathogen from feed have been confirmed in 12 regions of Ukraine. At

the same time, no cases were detected in the Autonomous Republic of Crimea, Volyn, Dnipropetrovsk, Zakarpattia, Kyiv, Kirovohrad, Mykolaiv, Poltava, Rivne, Kharkiv, Kherson, Cherkasy, and Chemivtsi regions (Fig. 1).

Analysis of botulism positive cases among various animal species and in feed shows that 1,551 positive cases, or 22.0% (BCI, 21.1–23.1%), were confirmed in animals, and the pathogen was isolated from feed in 5,480 cases, or 77.9% (BCI, 77.0–78.9%). In the total number of positive cases for the entire period analyzed, pigs dominated among animals – 645 or 41.6% (BCI, 39.1–44.1%), the combined category of other animals (cats, dogs, minks, foxes, wild rodents, poultry, etc.) – 446 cases or 28.8% (BCI, 26.5–31.1%), cattle – 339 or 21.9% (BCI, 19.8–24.0%), horses – 121 cases or 7.8% (BCI, 6.5–9.3%). No positive results were obtained from small ruminants. Quite high rates of positive results (for all groups) were observed in the period 2010–2017 (in 2010 – 828 cases, in 2011 – 794, in 2012 – 773, in 2013 – 658, in 2014 – 86, in 2015 – 1325, in 2016 – 1293, in 2017 – 1058 cases). During the period 2018–2024, a small number of studies were conducted, with 61 cases detected in 2018, 43 in 2019, 19 in 2020, 68 in 2021, 17 in 2022, and 13 positive cases in 2024. Thus, during the period 2010–2017, 6,815 samples tested positive for botulism

(BCI, 96.5–97.3%), and during 2018–2024, 221 samples tested positive (BCI, 2.7–3.6%, Table 1).

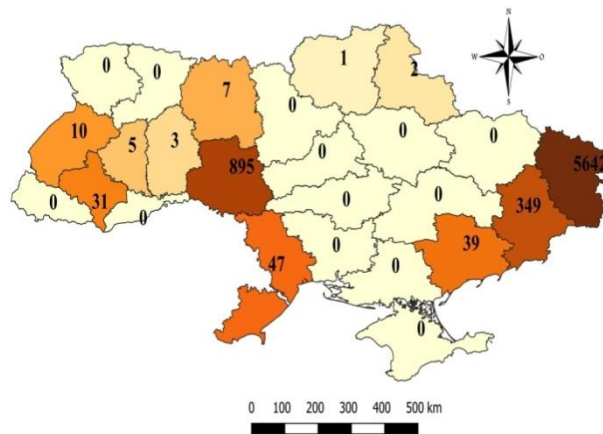


Fig. 1. Map of botulism cases among different animal species and positive samples of the pathogen isolated from feed (2010–2024)

Table 1

Number of botulism cases among different animal species and isolation of the pathogen from feed in Ukraine for the period 2010–2024

Years	Animal species					Feed	Total
	cattle	pigs	horses	small ruminants	others		
2010	6	16	–	–	114	692	828
2011	22	81	34	–	–	657	794
2012	73	87	11	–	210	392	773
2013	40	65	–	–	2	551	658
2014	41	–	–	–	41	4	86
2015	16	87	–	–	5	1212	1320
2016	29	166	68	–	11	1019	1293
2017	50	50	2	–	4	952	1058
2018	22	32	6	–	–	1	61
2019	11	17	–	–	15	–	43
2020	6	7	–	–	6	–	19
2021	23	37	–	–	8	–	68
2022	–	–	–	–	17	–	17
2023	–	–	–	–	–	–	–
2024	–	–	–	–	13	–	13
Total	339	645	121	–	446	5480	7031

It is interesting to note that between 2018 and 2024, only one positive sample for botulism was obtained in feed samples (in 2018), considering that the average number of positives for the previous eight years was 585.5 samples. We also note that no positive samples were found in samples from horses in 2019–2024. A similar situation is observed in studies among pigs and cattle in 2022–2024.

In general, the systematized results of the studies indicate that a significant amount of the botulism pathogen is found in feed. The incidence of botulism in animals is significantly lower. The number of detected cases of this disease in pigs (645) is more than 1.9 times higher than the number of cases in cattle (339) and more than 5.3 times higher than the number of positive cases in horses (121). A fairly significant number of positive cases (446) were also detected in the category “others”, which includes dogs, cats, rodents, etc. (Fig. 2).

In Ukraine, 1,070 cases of botulism were detected in humans during the period 2015–2024 (Table 2). The disease was registered in all regions of the country, with 9 to 121 cases in different regions. The number of botulism cases in humans has decreased slightly since 2020. The average for the period 2015–2019 was 128.5 cases per year (641 in total), and for the period 2020–2024 – 85.5 cases per year (429 in total). There has been a decrease in cases of this disease in humans by approximately 30%.

The highest number of positive cases during the period under review was observed in Kyiv (121 cases; BCI, 9.5–13.4%, with 72 cases registered in Kyiv city), Vinnytsia (77; BCI, 5.7–8.9%), and Zaporizhzhia (71; BCI, 5.2–8.3%) regions. Fewer cases were registered in Mykolaiv (9; BCI, 0.3–1.6%), Luhansk (10; BCI, 0.4–1.7%), Zakarpattia (12; BCI, 0.6–2.0%), and Sumy regions (17; BCI, 0.9–

2.5%). Most patients with a confirmed diagnosis were registered in the central and northwestern regions, and fewer in the southeastern regions (Fig. 3).

Analyzing the data presented in Fig. 4, it should be noted that the trends in the total number of cases among animals and the isolation of the botulism pathogen in feed do not correlate with cases of this disease in humans, especially in the period 2015–2017.

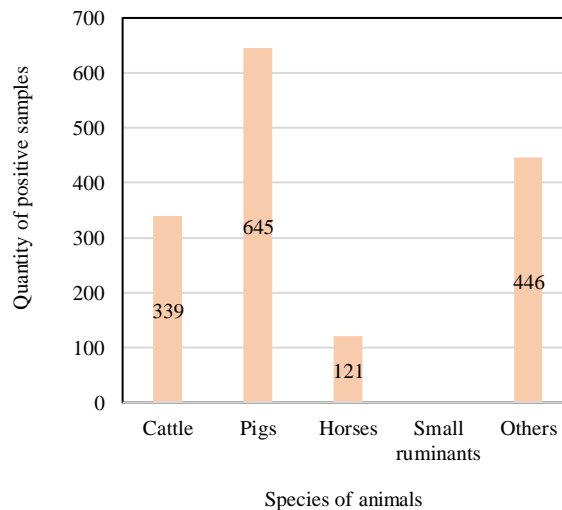


Fig. 2. Number of botulism cases among different animal species in Ukraine for the period 2010–2024

Table 2
Number of cases of botulism in humans in Ukraine (by region) for the period 2015–2024

Region	Years											total
	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024		
Vinnitsia	7	6	12	7	10	10	3	7	6	7	77	
Volyn	6	7	6	3	2	1	9	8	4	3	49	
Dnipropetrovsk	4	6	6	4	10	2	4	4	6	6	62	
Donetsk	4	2	4	9	5	3	1	–	2	–	30	
Zhytomyr	10	6	4	5	10	2	8	7	12	5	69	
Transcarpathian	4	1	2	–	–	1	–	1	2	1	12	
Zaporizhzhia	11	6	14	13	11	–	5	1	6	4	71	
Ivano-Frankivsk	10	4	3	1	9	–	1	5	5	1	39	
Kyiv	7	14	21	13	18	7	9	15	5	12	121	
Kirovograd	4	6	7	4	–	–	1	2	3	2	29	
Luhansk	1	3	1	4	–	1	–	–	–	–	10	
Lviv	15	4	9	2	2	3	8	5	1	9	58	
Mykolaiv	1	3	–	–	2	–	–	–	1	2	9	
Odessa	–	3	4	1	3	4	4	2	3	4	28	
Poltava	–	–	2	3	3	–	1	5	2	5	21	
Rivne	6	10	3	4	3	10	3	8	6	3	56	
Sumy	2	–	1	3	1	2	6	1	–	1	17	
Temopil	5	8	4	8	8	–	5	4	4	9	55	
Kharkiv	4	3	4	4	0	4	4	2	–	–	25	
Kherson	3	2	5	3	5	2	1	2	–	–	23	
Khmelnyskyi	5	7	11	8	9	4	6	5	3	3	61	
Cherkasy	15	5	9	9	7	2	7	2	4	9	69	
Chernivtsi	4	5	3	3	–	1	4	1	–	1	22	
Chernihiv	1	6	6	5	10	6	8	6	8	3	59	
Total	139	117	141	116	128	65	98	93	83	90	1070	

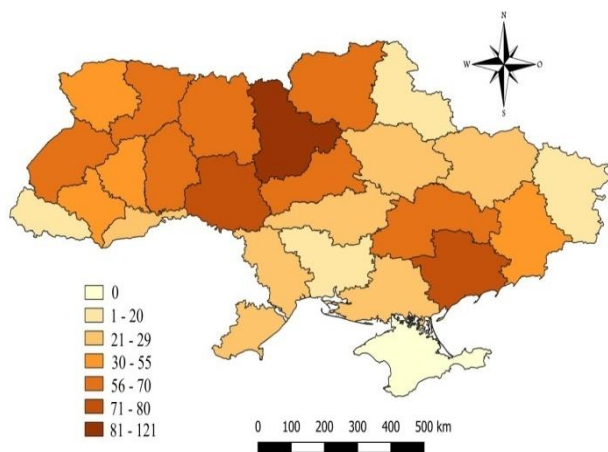


Fig. 3. Map of botulism cases among humans (2015–2024)

The number of cases in animals and the isolation of the pathogen in feed were 1,325, 1,293, and 1,058, respectively, while in the same years, 139, 117, and 141 cases of botulism were detected in humans. The difference is striking, as the number of botulism cases in animals and the amount isolated in feed is almost 10 times higher.

However, the situation changes radically if the pathogen isolated in feed is excluded from this component. In this case, we analyze the incidence figures in humans and animals, which are 108/139 in 2015, 117/274 in 2016, 141/106 in 2017, 116/60 in 2018, 128/43 in 2019 – 128/43, 2020 – 65/19, 2021 – 98/68, 2022 – 93/17, 2023 – 83/0, 2024 – 90/13. The total number of botulism cases in animals during the period under review (excluding the isolation of the pathogen from feed) is 708 among animals and 1070 among humans. The graphical trends in morbidity among animals practically overlap with the trends in morbidity among humans.

It is important to note that since 2018, the number of studies among animals and feed testing for the pathogen has decreased significantly. In fact, between 2018 and 2024, only one positive sample for botulism was identified during feed testing. In addition, for example, the number of positive cases among animals over the last 7 years (2018–2024) was 221, and over the previous 7 years (2011–2017) – 1,195, so we see a 5.4-fold decrease in the number of positive cases, as well as in the number of studies conducted on this disease.

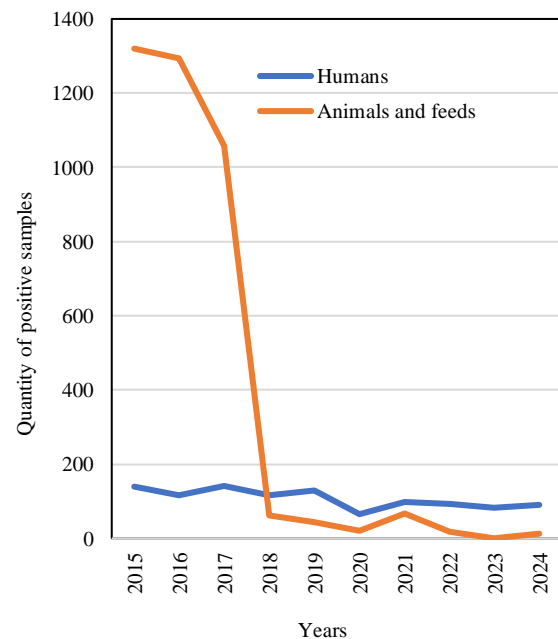


Fig. 4. Comparison of the number of botulism cases among humans and various animal species, including the pathogen isolated in feed in Ukraine for the period 2015–2024

Discussion

Currently, foodborne botulism is also a problem for domestic and wild animals, as outbreaks of this disease among farm animals and waterfowl often spread rapidly and lead to devastating epidemics due to the corresponding transmission cycles in the environment (Rawson et al., 2023). As in our case, we see a fairly significant correlation between cases of disease in animals and humans (coincidence of trend lines) in the graphs of morbidity of different species. Active bacterial growth leads to an increase in BoNT concentrations, and this toxin enters the bodies of animals, birds, and fish in various ways, and through them into the human body, causing fatal intoxication. Although it is true that humans can often be affected by homemade and

canned products that violate the relevant rules, *C. botulinum* remains an important pathogen because it is significant for public health. As we have found out, 1,070 people have contracted botulism in Ukraine over the past 10 years. There is also a potential risk of bioterrorism involving the use of this pathogen's toxin, but BoNT also has a wide range of pharmaceutical applications. Indeed, the use of BoNT in the medical and cosmetic industries stimulates research interest in this clostridiosis. However, the pathogen is quite dangerous, and working with a highly toxic neurotoxin and its inclusion in the list of selective biological agents requires an appropriate biosafety system, so work with it is limited to only a few authorized laboratories worldwide (Rawson et al., 2023).

In animals, botulism has been regulated since 2006 in most European countries, in domestic poultry, and later in wild birds and cattle. Until then, the pathogen was classified as a Category 1 health hazard for all susceptible species (Ministerial Order of July 29, 2013 Concerning the Definition of Category 1 and Category 2 Health Hazards for Animal Species). Subsequently, the Animal Health Law was adopted at the European level in 2016 (Regulation 2016/429 of the European Parliament and of the Council of March 9, 2016 on Transmissible Animal Diseases and Amending and Repealing Certain Acts in the Area of Animal Health), and the status of botulism changed, as the disease was no longer included in the list of pathogens that can be transmitted to animals or humans and to which appropriate prevention and control measures should apply. In our case, this is clearly reflected in the decrease in active research on this pathogen in feed and the reduction in the number of studies in animals. Indeed, between 2018 and 2024, only one positive sample with the pathogen was detected in feed in Ukraine, although in the previous eight years, the pathogen was isolated in almost 686 samples annually (5484 between 2010 and 2017). The number of positive samples for botulism among different animal species has also decreased significantly. For example, 1,281 positive results were obtained during the period 2010–2017 (more than 160 per year), and 220 during the period 2018–2024 (31 per year).

In Ukraine, during the period under review, the largest number of sick animals was registered among pigs – 645 cases (BCI, 39.1–44.1%), in the “others” category – 446 (which includes poultry, fur animals, domestic animals, and wild rodents) (BCI, 26.5–31.1%), among cattle – 339 (BCI, 19.8–24.0%), and 121 cases in horses (BCI, 6.5–9.3%).

At the same time, no positive results were obtained from small ruminants, which may be associated with a catastrophic decline in the population of these species (sheep and goats) on a national scale. Specialized literature also points to frequent infections in cattle (Rasetti-Escargueil et al., 2019), fur animals (Lindström et al., 2004; Le Bouquin et al., 2022) and horses (Galey, 2001). In France, for example, the disease is present in many species, is rare in humans, with an incidence of 10 cases per year, and is much more common in birds (wild and domestic) and cattle, which are the most affected categories of animals. Each year, an average of 10 outbreaks are recorded in cattle, 30 in poultry, and 20 in wild birds, each of which can affect several thousand birds (Rasetti-Escargueil et al., 2020). Among domestic birds, outbreaks of botulism are recorded in broilers, turkeys, pheasants and, to a lesser extent, ducks, laying hens (raised only on litter or free-range), geese, quails and guinea fowl (Le Maréchal et al., 2016, 2017; Ventujol et al., 2017). In most cases, botulism in humans and animals has different origins, and cross-transmission between animals and humans is a rare occurrence. However, due to the severity of this disease, human and animal botulism requires careful monitoring (Rasetti-Escargueil et al., 2019).

Wild birds, mainly waterfowl such as ducks, as well as farm-raised birds, often contract botulism. In Europe, North America, and Japan, frequent and significant outbreaks of botulism among waterfowl are regularly reported (Takeda et al., 2005; Defilippo et al., 2013). In Spain, 13 outbreaks of botulism among wild birds caused the death of about 20,000 birds, including more than 50 species between 1978 and 2008 (Vidal et al., 2013). In France, 129 outbreaks of botulism in wild birds and 396 in farm birds were recorded between 2000 and 2013 (Rasetti-Escargueil et al., 2019). During a study of 17 flocks affected by botulism, mortality ranged from 2.8% to 35.0%, each flock

numbering between 10,000 and 20,000 birds (Souillard et al., 2014). During major outbreaks of type E botulism in the Great Lakes (USA) between 2000 and 2008, the mortality was approximately 68,000 wild birds (Hannett et al., 2011; Rasetti-Escargueil et al., 2019). During a major outbreak of botulism in France, 80 out of 110 dairy cows died. The animals were fed grass silage contaminated with bird droppings (Relun et al., 2017). In Finland, 9 out of 90 cattle fed with unacidified silage contaminated with dead carrion (Myllykoski et al., 2009). In Brazil, a massive outbreak of type C botulism caused the death of 1,100 bulls from a herd of 1,700 that had been fed corn silage for two weeks. The source of the contamination of the corn silage was not identified (Guizelini et al., 2019).

The incidence rate in Europe is about 0.02 cases per 100,000 inhabitants, which is identical to the incidence rates in France (Therre, 1999; Le Bouquin et al., 2022). The countries with the highest number of confirmed cases are Italy, the United Kingdom, Poland, Romania, and France. In Italy, 466 cases of botulism were detected between 1986 and 2015: 93% were foodborne botulism, 6% were infant botulism, and 1% were wound botulism (Anniballi et al., 2017). In Turkey, 95 cases of botulism were detected between 1983 and 2017, with home-canned vegetables being the food category most responsible for these cases (Karsen et al., 2019). Infant botulism is the most common form of botulism in the United States and accounts for 80% of reported cases of infant botulism worldwide since this form of the disease was first identified in 1976. The average annual incidence rate is 2.1 cases per 100,000 births (Le Bouquin et al., 2022). Between 1976 and 2016, 1,345 cases (6.5 cases per 100,000 births per year) caused by types A, B, Ba, Bf, and F were detected in California (Panditrao et al., 2020). The average annual incidence rate was calculated at 4.3 cases per million births in Canada during the period 1979–2019 (Harris et al., 2021). An analysis of toxin types found in France confirmed the predominance of types A and B in human botulism both in cases of food poisoning and in cases of infant botulism and exclusively type F (Castor et al., 2015; Tréhard et al., 2016). Internationally, the types of botulism that cause human cases are types A and B, followed by E and, occasionally, F. A meta-analysis of outbreaks covering 197 outbreaks of foodborne botulism (almost half of which were outbreaks in the United States) identified botulism types A, B, E, and F as the causative agent of botulism in 34, 16, 17, and 1% of outbreaks, respectively (Fleck-Derderian et al., 2017). Botulism B is the most common type of botulism in France, as well as in Poland, where type B accounted for 83% of cases in 2016 (Czerwiński et al., 2018). In Italy, from 1986 to 2015, botulism type B was responsible for 79.1% of cases (261/330), followed by botulism type A (9.7%, 32/330), while botulism type F, Ab, and Bf accounted for 0.3 (1/330), 1.5 (5/330), and 0.6% (2/330) of all cases, respectively (Anniballi et al., 2017). In Ukraine, the most common types are botulism type B (59.64%), E (25.47%), and A (7.97%), while cases associated with type C botulism are very rare (0.56%) and are only suspected (Semenko et al., 2021). In North America, outbreaks of foodborne botulism occur due to vegetables (home-canned foods), but this mainly concerns type E botulism originating from fish or marine mammals prepared in indigenous communities using traditional methods (e.g., fish fermentation). Similarly, in various Asian countries, outbreaks usually occur due to traditional food preparation (Kenri et al., 2014; Khorasan et al., 2020).

European countries report the involvement of BoNT C and D in botulism outbreaks in animals (Woudstra et al., 2012; Fillo et al., 2021). Although most bird species are experimentally susceptible to various BoNTs, the only BoNTs that naturally contribute to outbreaks in birds are BoNT C, D, or their variants C/D and D/C, BoNT E, and, much less frequently, BoNT A. BoNT C, D, or their variants C/D and D/C are predominant in both wild and domestic species. BoNT E is less common and regularly causes sporadic cases or epizootics in wild fish-eating birds in northern regions, but rarely causes epizootics in agricultural species. Type A botulism has been reported only a few times in the United States among bird species, including the death of gulls in the Klamath River basin in California (Rogers et al., 2021), and appears to be extremely rare on farms (outbreak on a broiler farm in the US (Le Bouquin et al., 2022). In France, only BoNT D/C and C

have been detected in recent years during outbreaks of botulism in cattle. In Europe, BoNT D/C is currently responsible for most cases of disease in cattle (Nakamura et al., 2013; Fillo et al., 2021). Very rare cases of BoNT A were reported in the mid-20th century in France (Le Bouquin et al., 2022), as cited by the French Agency for Food Safety and Animal Health, in zebu (*Bos indicus*) in Brazil (Schocken-Iurrino et al., 1990), in dairy cows in Egypt in 1976 (Rasetti-Escargueil et al., 2019), and most recently in New York State in the United States (Frye et al., 2020). Type B outbreaks have also been described in the literature among dairy herds: in the United States in 1984, 1992, and 2001, in Israel in 2000 (Divers et al., 1986; Wilson et al., 1995; Kelch et al., 2000; Yeruham et al., 2003) and in the Netherlands in approximately 30 dairy herds in 1976 and 1977 in connection with the inclusion of contaminated brewers' grains in the feed ration (Notermans et al., 1985).

In Ukraine, between 1955 and 2018, 8,614 cases of botulism were registered in humans (Semenko et al., 2021). The authors indicate that 659 fatal cases were identified. Homemade canned meat and fish were the main causes of botulism in Ukraine. Our research showed that the total number of botulism cases in animals (excluding the isolation of the pathogen from feed) between 2015 and 2024 was 708 among animals and 1,070 among humans. The graphical trends in morbidity among animals practically overlap with the trends in human morbidity. However, "animal" botulism has a negligible effect on the manifestation of botulism in humans. The graphical trend in human morbidity was practically unaffected by the improvement in the epizootic situation with this disease in animal husbandry.

Botulism in animals occurs either as a result of preformed BoNT entering the body with food, or as a result of intestinal colonization and subsequent toxin production in the intestine. The main source of botulism due to intoxication in animals is the carcasses of small animals (rodents, birds, cats) in food or decomposing carcasses chewed by cattle with phosphorus deficiency (Galey et al., 2000; Myllykoski et al., 2009). BoNT production can also occur in fermented grains and poorly preserved silage with a pH > 4.5 (Guizelini et al., 2019). Botulism through intestinal colonization is a common form in farm birds and cattle. In most outbreaks of botulism in cattle or poultry, BoNT was not detected in silage or commercial cattle feed based on dry, ground grains containing *C. botulinum* spores, which were responsible for botulism (Krüger et al., 2014; Relun et al., 2017). In addition, cases of botulism are usually observed up to two weeks after the removal of contaminated food, which supports infection by intestinal colonization. Young animals appear to be more susceptible to botulism through intestinal colonization than humans. Thus, botulism through intestinal colonization is mostly found in foals, while foodborne botulism due to intoxication is more common in adult horses (Johnson et al., 2010).

Therefore, in many countries around the world, as in Ukraine, human botulism is caused by the consumption of ham (pig farming) and canned vegetables. The latter prompts the collection of information and control of the food industry and livestock sector, as well as active monitoring of this pathogen in the environment. Such work will be aimed at preventing this disease in animals and humans, and in this regard, it is necessary to work more actively to improve data collection and monitoring of food products, feed, and environmental pollution (Bordier et al., 2020). In this regard (feed testing), Ukraine is somewhat lagging behind, as 5,480 cases of feed contamination were detected between 2010 and 2024, but only one sample was tested between 2018 and 2024.

In Ukraine, botulism in humans is mainly caused by traditions and socio-economic factors. In conditions of declining living standards and high meat prices, home canning is widely practiced, which is the most common cause of human infection. Even our domestic researchers note that the elimination of human botulism in Ukraine is only possible if home canning is completely stopped and the production and sale of commercial canned foods is subject to state control (Semenko et al., 2021).

Botulism is included in the European list B of Annex I to the Directive on zoonoses (Directive 2003/99/EC of the European Parliament). The observation and study of botulism, BoNT, and BoNT-producing clostridia logically falls under the One Health concept. While

botulism is notifiable for humans in Europe, this does not systematically apply to animals. In France, botulism is a notifiable disease in both humans and animals, regardless of the species affected, which allows an overall picture to be obtained. Cases and outbreaks of botulism are carefully monitored through a passive surveillance system based on individual cases. This is the first step in applying a "One Health" approach to disease control by combining animal and human surveillance. In most cases, surveillance systems continue to be developed and operated within a highly sectoral approach (Baum et al., 2016). However, in order to be effective, the management of complex health problems must move from isolated, sectoral, and linear approaches to systemic and transdisciplinary approaches to health (Bordier et al., 2020). Human botulism was often caused by the consumption of ham (the pig farming sector and it is quite alarming that the largest number of sick animals in Ukraine during the period under review was registered in pigs – 645 cases) and canned vegetables, which indicates the importance of collecting surveillance data from the food industry, livestock sectors, as well as monitoring this pathogen in the environment. These results show that even if surveillance is implemented for both human and animal health, progress is still needed to improve data collection and surveillance of food, feed, and environmental contamination.

Conclusion

During the period under review (2015–2024), 1,551 cases of botulism in animals were registered in Ukraine, and the pathogen was detected in 5,480 feed samples. There were 339 cases in cattle, 645 in pigs, 121 in horses, and 446 in other animal categories. The highest number of cases of botulism in feed and in various animal species was recorded in the Luhansk (5,642), Vinnytsia (895), and Donetsk (349) regions. Between 2015 and 2024, 1,070 cases of botulism in humans were registered. The highest incidence rates in humans were recorded in the Kyiv (121), Vinnytsia (77), and Zaporizhzhia (71) regions.

Although the graphical trends in human incidence somewhat coincide with those in animal incidence, the situation in animal husbandry does not have a significant impact on human incidence. In addition, after 2017, the annual incidence among humans decreased by approximately 30%, and the incidence among animals decreased by more than 80%. It should be noted that feed is not currently tested for the presence of the pathogen either. Between 2010 and 2017, 5,479 samples of feed contaminated with the pathogen were detected, and between 2018 and 2024, only one sample was detected. This situation raises concerns about the decrease in the number of biological materials from animals suspected of having botulism being tested and the testing of significantly fewer feed samples. Accordingly, this component requires the restoration of stricter control.

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References

- Annibaldi, F., Auricchio, B., Fiore, A., Lonati, D., Locatelli, C. A., Lista, F., Fillo, S., Mandarino, G., & De Medici, D. (2017). Botulism in Italy, 1986 to 2015. *European Communicable Disease Bulletin*, 22(24), 30550.
- Annibaldi, F., Lonati, D., Fiore, A., Auricchio, B., De Medici, D., & Locatelli, C. A. (2014). New targets in the search for preventive and therapeutic agents for botulism. *Expert Review of Anti-Infective Therapy*, 12(9), 1075–1086.
- Arnon, S. S., Schechter, R., Inglesby, T. V., Henderson, D. A., Bartlett, J. G., Ascher, M. S., Eitzen, E., Fine, A. D., Hauer, J., Layton, M., Lillibridge, S., Osterholm, M. T., O'Toole, T., Parker, G., Perl, T. M., Russell, P. K., Swerdlow, D. L., & Tonat, K. (2001). Botulinum toxin as a biological weapon: Medical and public health management. *Journal of the American Medical Association*, 285(8), 1059–1070.
- Baum, S. E., Machalaba, C., Daszak, P., Salerno, R. H., & Karesh, W. B. (2016). Evaluating one health: Are we demonstrating effectiveness? *One Health*, 3, 5–10.
- Bordier, M., Uea-Anuwong, T., Binot, A., Hendrikx, P., & Goutard, F. L. (2020). Characteristics of One Health surveillance systems: A systematic literature review. *Preventive Veterinary Medicine*, 181, 104560.

- Castor, C., Mazuet, C., Saint-Leger, M., Vygen, S., Coutureau, J., Durand, M., Popoff, M. R., & Jourdan Da Silva, N. (2015). Cluster of two cases of botulism due to *Clostridium baratii* type F in France, November 2014. *European Communicable Disease Bulletin*, 20(6), 21031.
- Czerwiński, M., Czarkowski, M. P., & Kondej, B. (2018). Foodborne botulism in Poland in 2016. *Przeegląd Epidemiologiczny*, 72(2), 149–155.
- Defilippo, F., Luppi, A., Maioli, G., Marzi, D., Fontana, M. C., Paoletti, F., Bonilauri, P., Dottori, M., & Meriardi, G. (2013). Outbreak of type C botulism in birds and mammals in the Emilia Romagna Region, Northern Italy. *Journal of Wildlife Diseases*, 49(4), 1042–1046.
- Divers, T. J., Bartholomew, R. C., Messick, J. B., Whitlock, R. H., & Sweeney, R. W. (1986). *Clostridium botulinum* type B toxicosis in a herd of cattle and a group of mules. *Journal of the American Veterinary Medical Association*, 188(4), 382–386.
- Duncan, R. M., & Jensen, W. L. (1976). A relationship between avian carcasses and living invertebrates in the epizootiology of avian botulism. *Journal of Wildlife Diseases*, 12(1), 116–126.
- Espelund, M., & Klavness, D. (2014). Botulism outbreaks in natural environments – an update. *Frontiers in Microbiology*, 5, 287.
- Fillo, S., Giordani, F., Tonon, E., Drigo, I., Anselmo, A., Fortunato, A., Lista, F., & Bano, L. (2021). Extensive genome exploration of *Clostridium botulinum* group III field strains. *Microorganisms*, 9(11), 2347.
- Fleck-Deerderian, S., Shankar, M., Rao, A. K., Chatham-Stephens, K., Adjei, S., Sobel, J., Meltzer, M. I., Meaney-Delman, D., & Pillai, S. K. (2017). The epidemiology of foodborne botulism outbreaks: A systematic review. *Clinical Infectious Diseases*, 66(1), 73–81.
- Frye, E. A., Egan, C., Perry, M. J., Crouch, E. E., Burbank, K. E., & Kelly, K. M. (2020). Outbreak of botulism type A in dairy cows detected by MALDI-TOF mass spectrometry. *Journal of Veterinary Diagnostic Investigation*, 32(5), 722–726.
- Galey, F. D. (2001). Botulism in the horse. *The Veterinary Clinics of North America. Equine Practice*, 17(3), 579–588.
- Galey, F. D., Terra, R., Walker, R., Adaska, J., Etchebarne, M. A., Puschner, B., Fisher, E., Whitlock, R. H., Rocke, T., Willoughby, D., & Tor, E. (2000). Type C botulism in dairy cattle from feed contaminated with a dead cat. *Journal of Veterinary Diagnostic Investigation*, 12(3), 204–209.
- Guizelini, C. C., Lemos, R. A. A., de Paula, J. L. P., Pupin, R. C., Gomes, D. C., Barros, C. S. L., Neves, D. A., Alcântara, L. O. B., Silva, R. O. S., Lobato, F. C. F., & Martins, T. B. (2019). Type C botulism outbreak in feedlot cattle fed contaminated corn silage. *Anaerobe*, 55, 103–106.
- Hannett, G. E., Stone, W. B., Davis, S. W., & Wroblewski, D. (2011). Biodiversity of *Clostridium botulinum* type E associated with a large outbreak of botulism in wildlife from Lake Erie and Lake Ontario. *Applied and Environmental Microbiology*, 77(3), 1061–1068.
- Harris, R., Tchao, C., Prystajecy, N., Cutler, J., & Austin, J. W. (2021). A summary of surveillance, morbidity and microbiology of laboratory-confirmed cases of infant botulism in Canada, 1979–2019. *Canada Communicable Disease Report*, 47(78), 322–328.
- Hill, K. K., & Smith, T. J. (2013). Genetic diversity within *Clostridium botulinum* serotypes, botulinum neurotoxin gene clusters and toxin subtypes. *Current Topics in Microbiology and Immunology*, 364, 1–20.
- Janik, E., Ceremuga, M., Saluk-Bijak, J., & Bijak, M. (2019). Biological toxins as the potential tools for bioterrorism. *International Journal of Molecular Sciences*, 20(5), 1181.
- Johnson, A. L., McAdams, S. C., & Whitlock, R. H. (2010). Type A botulism in horses in the United States: A review of the past ten years (1998–2008). *Journal of Veterinary Diagnostic Investigation*, 22(2), 165–173.
- Johnson, A. L., McAdams-Gallagher, S. C., & Aceto, H. (2016). Accuracy of a mouse bioassay for the diagnosis of botulism in horses. *Journal of Veterinary Internal Medicine*, 30(4), 1293–1299.
- Kalb, S. R., Baudys, J., Raphael, B. H., Dykes, J. K., Lúquez, C., Maslanka, S. E., & Barr, J. R. (2015). Functional characterization of botulinum neurotoxin serotype H as a hybrid of known serotypes F and A (BoNT F/A). *Analytical Chemistry*, 87(7), 3911–3917.
- Karsen, H., Ceylan, M. R., Bayındır, H., & Akdeniz, H. (2019). Foodborne botulism in Turkey, 1983 to 2017. *Infectious Diseases*, 51(2), 91–96.
- Kelch, W. J., Kerr, L. A., Pringle, J. K., Rohrbach, B. W., & Whitlock, R. H. (2000). Fatal *Clostridium botulinum* toxicosis in eleven Holstein cattle fed round bale barley haylage. *Journal of Veterinary Diagnostic Investigation*, 12(5), 453–455.
- Kenri, T., Sekizuka, T., Yamamoto, A., Iwaki, M., Komiya, T., Hatakeyama, T., Nakajima, H., Takahashi, M., Kuroda, M., & Shibayama, K. (2014). Genetic characterization and comparison of *Clostridium botulinum* isolates from botulism cases in Japan between 2006 and 2011. *Applied and Environmental Microbiology*, 80(22), 6954–6964.
- Khorasan, M. R. M., Rahbar, M., Bialvaei, A. Z., Gouya, M. M., Shahcheraghi, F., & Eshrafi, B. (2020). Prevalence, risk factors, and epidemiology of food-borne botulism in Iran. *Journal of Epidemiology and Global Health*, 10(4), 288–292.
- Krüger, M., Neuhaus, J., Herrenthey, A. G., Gökce, M. M., Schrödl, W., & Shehata, A. A. (2014). Chronic botulism in a Saxony dairy farm: Sources, predisposing factors, development of the disease and treatment possibilities. *Anaerobe*, 28, 220–225.
- Le Bouquin, S., Lucas, C., Souillard, R., Le Maréchal, C., Petit, K., Kooh, P., Jourdan-Da Silva, N., Meurens, F., Guillier, L., & Mazuet, C. (2022). Human and animal botulism surveillance in France from 2008 to 2019. *Frontiers in Public Health*, 10, 1003917.
- Le Maréchal, C., Ballan, V., Rouxel, S., Bayon-Auboyer, M. H., Baudouard, M. A., Morvan, H., Houard, E., Požvevara, T., Souillard, R., Woudstra, C., Le Bouquin, S., Fach, P., & Chemaly, M. (2016). Livers provide a reliable matrix for real-time PCR confirmation of avian botulism. *Anaerobe*, 38, 7–13.
- Le Maréchal, C., Rouxel, S., Ballan, V., Houard, E., Požvevara, T., Bayon-Auboyer, M. H., Souillard, R., Morvan, H., Baudouard, M. A., Woudstra, C., Mazuet, C., Le Bouquin, S., Fach, P., Popoff, M., & Chemaly, M. (2017). Development and validation of a new reliable method for the diagnosis of avian botulism. *PLoS One*, 12(1), e0169640.
- Lindström, M., Nevas, M., Kurki, J., Sauna-aho, R., Latvala-Kiesilä, A., Pölonen, I., & Korkeala, H. (2004). Type C botulism due to toxic feed affecting 52,000 farmed foxes and minks in Finland. *Journal of Clinical Microbiology*, 42(10), 4718–4725.
- Maslanka, S. E., Lúquez, C., Dykes, J. K., Tepp, W. H., Pier, C. L., Pellett, S., Raphael, B. H., Kalb, S. R., Barr, J. R., Rao, A., & Johnson, E. A. (2016). A novel botulinum neurotoxin, previously reported as serotype H, has a hybrid-like structure with regions of similarity to the structures of serotypes A and F and is neutralized with serotype A antitoxin. *The Journal of Infectious Diseases*, 213(3), 379–385.
- Myllykoski, J., Lindström, M., Keto-Timonen, R., Söderholm, H., Jakala, J., Kallio, H., Sukura, A., & Korkeala, H. (2009). Type C bovine botulism outbreak due to carcass contaminated non-acidified silage. *Epidemiology and Infection*, 137(2), 284–293.
- Nakamura, K., Kohda, T., Seto, Y., Mukamoto, M., & Kozaki, S. (2013). Improved detection methods by genetic and immunological techniques for botulinum C/D and D/C mosaic neurotoxins. *Veterinary Microbiology*, 162(2–4), 881–890.
- Notermans, S. H., Havelaar, A. H., Dufrenne, J. B., & Oosterom, J. (1985). Voorkomen van *Clostridium botulinum* op rundveehouderij bedrijven [Incidence of *Clostridium botulinum* on cattle farms]. *Tijdschrift Voor Diergeneeskunde*, 110(5), 175–180.
- Panditrao, M. V., Dabritz, H. A., Kazerouni, N. N., Damus, K. H., Meisinger, J. K., & Armon, S. S. (2020). Descriptive epidemiology of infant botulism in California: The first 40 years. *The Journal of Pediatrics*, 227, 247–257.
- Peck, M. W. (2009). Biology and genomic analysis of *Clostridium botulinum*. *Advances in Microbial Physiology*, 55, 183–320.
- Peck, M. W., Smith, T. J., Anniballi, F., Austin, J. W., Bano, L., Bradshaw, M., Cuervo, P., Cheng, L. W., Derman, Y., Dorner, B. G., Fisher, A., Hill, K. K., Kalb, S. R., Korkeala, H., Lindström, M., Lista, F., Lúquez, C., Mazuet, C., Pirazzini, M., Popoff, M. R., & Stringer, S. C. (2017). Historical perspectives and guidelines for botulinum neurotoxin subtype nomenclature. *Toxins*, 9(1), 38.
- Popoff, M. R. (1989). *Revue scientifique et technique. International Office of Epizootics*, 8(1), 129–145.
- Rasetti-Escargueil, C., Lemichez, E., & Popoff, M. R. (2019). Public health risk associated with botulism as foodborne zoonoses. *Toxins*, 12(1), 17.
- Rasetti-Escargueil, C., Lemichez, E., & Popoff, M. R. (2020). Toxemia in human naturally acquired botulism. *Toxins*, 12(11), 716.
- Rawson, A. M., Dempster, A. W., Humphreys, C. M., & Minton, N. P. (2023). Pathogenicity and virulence of *Clostridium botulinum*. *Virulence*, 14(1), 2205251.
- Relun, A., Dorso, L., Douart, A., Chartier, C., Guatteo, R., Mazuet, C., Popoff, M. R., & Assié, S. (2017). A large outbreak of bovine botulism possibly linked to a massive contamination of grass silage by type D/C *Clostridium botulinum* spores on a farm with dairy and poultry operations. *Epidemiology and Infection*, 145(16), 3477–3485.
- Rogers, K. H., Viera, O. G., Uzal, F. A., Peronne, L., & Mete, A. (2021). Mortality of western gulls (*Larus occidentalis*) associated with botulism type A in coastal Southern California, USA. *Journal of Wildlife Diseases*, 57(3), 657–661.
- Rossetto, O., Pirazzini, M., Fabris, F., & Montecucco, C. (2021). Botulinum neurotoxins: Mechanism of action. *Handbook of Experimental Pharmacology*, 263, 35–47.
- Schocken-Turrino, R. P., Avila, F. A., Berchielli, S. C., & Nader Filho, A. (1990). First case of type A botulism in zebu (*Bos indicus*). *The Veterinary Record*, 126(9), 217–218.
- Semenko, N., Mokhort, H., Sokolovska, O., Kolesnikova, I., Kuzin, I., & Saylors, K. (2021). Foodborne botulism in Ukraine from 1955 to 2018. *Foodborne Pathogens and Disease*, 18(4), 260–266.

- Smith, T. J., Lou, J., Geren, I. N., Forsyth, C. M., Tsai, R., Laporte, S. L., Tepp, W. H., Bradshaw, M., Johnson, E. A., Smith, L. A., & Marks, J. D. (2005). Sequence variation within botulinum neurotoxin serotypes impacts antibody binding and neutralization. *Infection and Immunity*, 73(9), 5450–5457.
- Sobel, J. (2005). Botulism. *Clinical Infectious Diseases*, 41(8), 1167–1173.
- Souillard, R., Woudstra, C., Le Maréchal, C., Dia, M., Bayon-Auboyer, M. H., Chemaly, M., Fach, P., & Le Bouquin, S. (2014). Investigation of *Clostridium botulinum* in commercial poultry farms in France between 2011 and 2013. *Avian Pathology*, 43(5), 458–464.
- Takeda, M., Tsukamoto, K., Kohda, T., Matsui, M., Mukamoto, M., & Kozaki, S. (2005). Characterization of the neurotoxin produced by isolates associated with avian botulism. *Avian Diseases*, 49(3), 376–381.
- Therre, H. (1999). Botulism in the European Union. *European Communicable Disease Bulletin*, 4(1), 2–7.
- Tréhard, H., Poujol, I., Mazuet, C., Blanc, Q., Gillet, Y., Rossignol, F., Popoff, M. R., & Jourdan Da Silva, N. (2016). A cluster of three cases of botulism due to *Clostridium baratii* type F, France, August 2015. *European Communicable Disease Bulletin*, 21(4), 10.2807/1560-7917.ES.2016.21.4.30117.
- Ventujol, A., Decors, A., Le Maréchal, C., Toux, J. Y., Allain, V., & Mazuet, C. (2017). Le botulisme aviaire en France: Etude des cas signalés dans la faune sauvage et dans les élevages par deux réseaux de surveillance entre 2000 et 2013. *Epidémiologie Sante Animale*, 72, 85–102.
- Vidal, D., Anza, I., Taggart, M. A., Pérez-Ramírez, E., Crespo, E., Hofle, U., & Mateo, R. (2013). Environmental factors influencing the prevalence of a *Clostridium botulinum* type C/D mosaic strain in nonpermanent Mediterranean wetlands. *Applied and Environmental Microbiology*, 79(14), 4264–4271.
- Wilson, R. B., Boley, M. T., & Corwin, B. (1995). Presumptive botulism in cattle associated with plastic-packaged hay. *Journal of Veterinary Diagnostic Investigation*, 7(1), 167–169.
- Woudstra, C., Skarin, H., Anniballi, F., Fencia, L., Bano, L., Drigo, I., Koene, M., Bayon-Auboyer, M. H., Buffereau, J. P., De Medici, D., & Fach, P. (2012). Neurotoxin gene profiling of *Clostridium botulinum* types C and D native to different countries within Europe. *Applied and Environmental Microbiology*, 78(9), 3120–3127.
- Yeruham, I., Elad, D., Avidar, Y., Grinberg, K., Tiomkin, D., & Monbaz, A. (2003). Outbreak of botulism type B in a dairy cattle herd: Clinical and epidemiological aspects. *The Veterinary Record*, 153(9), 270–272.
- Zhang, S., Masuyer, G., Zhang, J., Shen, Y., Lundin, D., Henriksson, L., Miyashita, S. I., Martínez-Carranza, M., Dong, M., & Stenmark, P. (2017). Identification and characterization of a novel botulinum neurotoxin. *Nature Communications*, 8, 14130.