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CLOSTRIDIA SPREAD IN LIVESTOCK ANIMALS: SITUATION AND INITIATIVES

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PRESENTATION STRUCTURE

INTRODUCTION

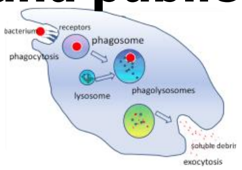
CLOSTRIDIUM PERFRINGENS **: An emerging threat for animal and public health**

C. PERFRINGENS SPREAD IN:

- ❑**RUMINANTS**
- ❑**PIGS**
- ❑**POULTRY**

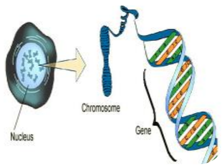
RISK FOR PUBLIC HEALTH ?


EPIDEMIOLOGY



PATHOGENICITY


STATISTICS



MOLECULAR CHARACTERIZATION

CLOSTRIDIUM DIFFICILE **– emerging risk associated with animals**

EPIDEMIOLOGY AND TOXIGENICITY OF COMMON CLOSTRIDIUM DIFFICILE RIBOTYPES IN:

- ❑**RUMINANTS**
- ❑**PIGS**
- ❑**POULTRY**

CURRENT SITUATION IN ROMANIA

CONCLUSIONS

INTRODUCTION

- Heterogeneous group of environmental bacteria but can be found also as pathogens in humans and animals (Liu, 2011).
- In this particular bacteria genre there are about 15 pathogenic species which are the cause of the most dangerous toxins known to man (Broda, 2000).
- Although animals are the main reservoirs for Clostridium species, a number of food products have been reported to be the cause of the infection if proper hygiene is not practiced along their processing technology (Rodriguez et al., 2016).
- The contamination occurs by ingestion of food with clostridia endospores which are known to be highly resistant to standard cooking and food processing measures (Jöbstl et al., 2010).

C. perfringens: gas gangrene; food poisoning

C. tetani: tetanus

C. botulinum: botulism

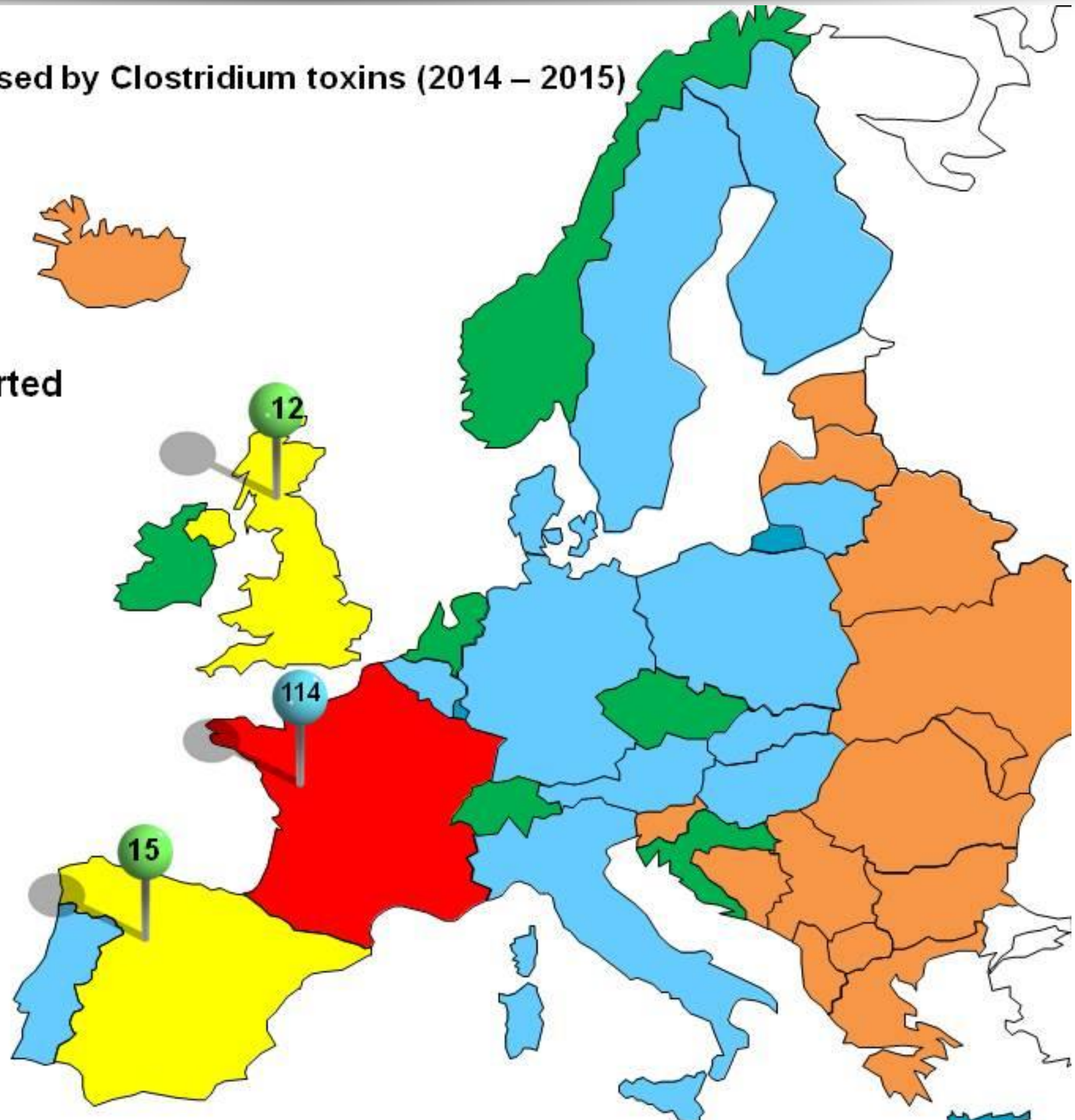
C. difficile: pseudomembranous colitis

INTRODUCTION

Specie	Human disease	Frequency
C. difficile	Antibiotic – associated diarrhea, pseudo membranous colitis	common
C. perfringens	Soft tissue infections (i.e., cellulitis, suppurative myositis, myonecrosis or gas gangrene), food poisoning, enteritis necroticans, septicemia	common
C. septicum	Gas gangrene, septicemia	uncommon
C. tertium	Opportunistic infections	Uncommon
C. botulinum	Botulism	Uncommon
C. tetani	Tetanus	Uncommon
C. barati	Botulism	Rare
C. butyricum	Botulism	Rare
C. novyi	Gas gangrene	Rare
C. sordellii	Gas gangrene	Rare

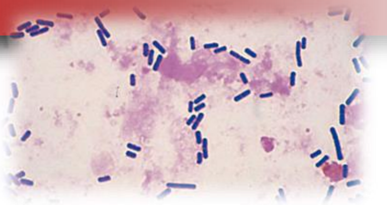
Food-borne outbreaks caused by Clostridium toxins (2014 – 2015)

- > 20
- > 10
- < 10
- No outbreaks reported
- No data



Based on EFSA (2015)

CLOSTRIDIUM PERFRINGENS: CHARACTERISTICS



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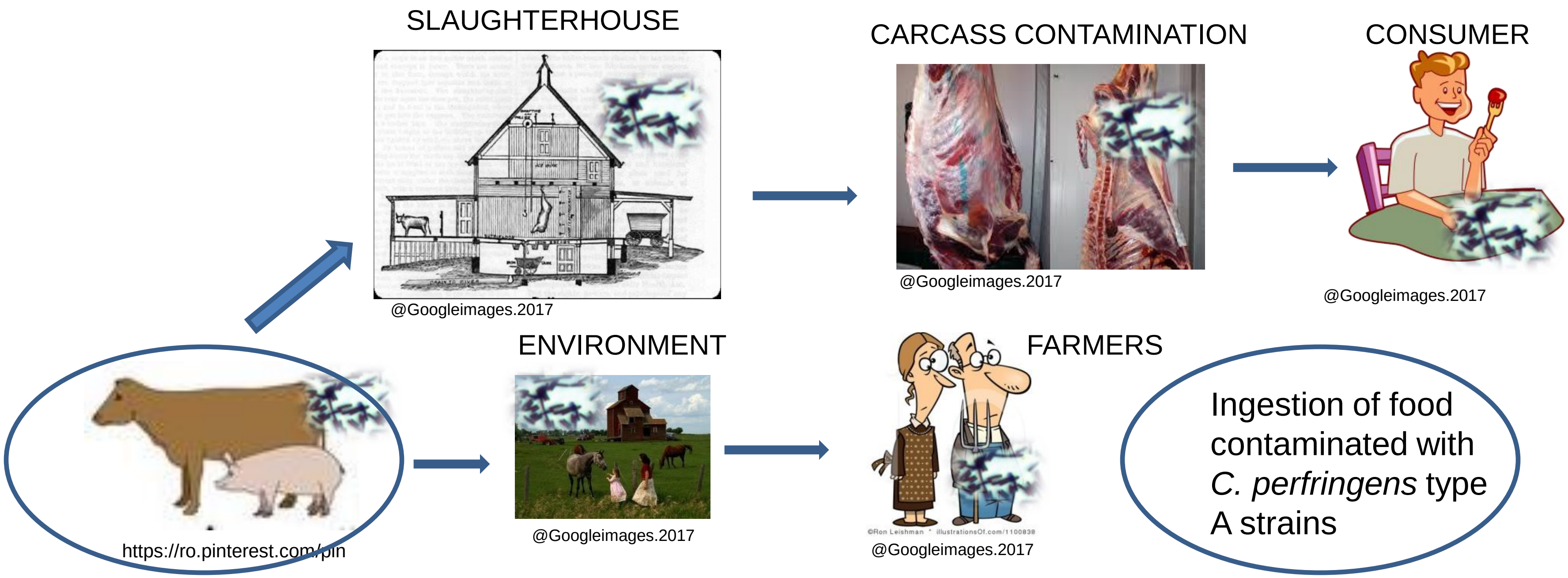
- Gram-positive, rod-shaped, spore forming, oxygen-tolerant anaerobe

TOXINOTYPE	MAJOR TOXIN	GENOTYPE	HUMAN DISEASE	GI DISEASE IN
A	α-β	*cpa*	gas gangrene	horse, pig
		cpa, cpb, cpe	gastrointestinal disease	fowl, yellow lamb disease
B	α-β-ε	*cpa, cpb1, etx*		sheep, horses, cows
C	α-β	*cpa, cpb1*	necrotizing enteritis	neonatal necrotic enteritis of (horse, cattle, sheep, pigs)
D	α-ε	*cpa, etx*		enterotoxemia of sheep and goats
E	α-ι	*cpa, iap*	No known association with human disease;	no confirmed GID of cattle, sheep and rabbits

C. PERFRINGENS: An emerging threat for animal and public health

- *C. perfringens* type A food poisoning is one of the most common food-borne illnesses in industrially developed countries (Fafangel et al., 2015).

- In USA, *C. perfringens* is the third most common cause of food-borne illness (10%) of cases (Wen, 20014). In EU, England and Wales reported 81 outbreaks, affecting almost 3000 persons (Fafangel et al., 2015).



CLOSTRIDIUM PERFRINGENS SPREAD IN RUMINANTS

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- *C. perfringens* type A has been and is still frequently blamed for enteritis, abomasitis and/or enterotoxemia in cattle (Fohler et al., 2016).

- Studies on the occurring types of *C. perfringens* in cattle including all toxin genes **are rare.**

- **most studies** on the epidemiology of enterotoxigenic *C. perfringens* in ruminants (dairy cattle) **did not recover isolates positive for *cpe*** (incriminated for causing illnesses in humans), and therefore all presumed that they are not a primary source for food poisoning strains (Fohler et al., 2016; Lindstrom et al., 2011).

Country	No. of farms / no. of isolates	Genotype (no. of isolates)	Type	Reference
Germany	139 /662	Cpa (435) **Cpa + Cpb (220)** Cpa + Cpe (5)	A	Fohler et al. (2016)
		Cpa + etx (2)	D	
Belgium	14/87	Cpa +cpb2 (87)	A	Lebrun et al. (2007)
Canada	75/74	Cpa (64) **Cpa +cpb2 (10)**	A	Schlegel et al. (2012)
USA	7/241	Cpa (68) Cpa + Cpb (6) **Cpa + Cpb2 (164)** Cpa +Cpe (11)	A	Gurjar et al. (2008)
		Cpa +ia (6)	E	
		Cpa +etx (4)	D	

CLOSTRIDIUM PERFRINGENS SPREAD IN RUMINANTS


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➢C. perfringens is **responsible for enterotoxemia in sheep and goat** - major economic obstacle facing developing countries attributable to the high fatality rate, decreased productivity, and increased treatment costs (Greco et al., 2006).

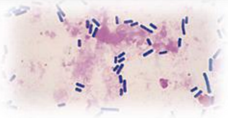
➢**C. perfringens type A** causes yellow lamb disease, a rare form of acute enterotoxemia in lambs.

➢ **C. perfringens type B** infection - lamb dysentery and/or hemorrhagic enteritis, sudden death is the main feature of this form.

➢ **C. perfringens type D** infection - pulpy kidney disease.

Country	No. of farms / no. of isolates	Genotype (no. of isolates)	Type	Reference
Turkey	298/113	Cpa (33) Cpa . Etx (11) cpa, cpb (2)	A D C	Kalender et al. (2005)
	149/13	Cpa (10) Cpa , Etx (2) cpa, cpb (1)	A D C	Hadimli et al. (2012)
Saudi Arabia	?/34	Cpa (18) Cpa +cpb (2) cpa, cpb1 (6) Cpa (8)	A B C D	Fayez et al. (2013)
Belgium	48/63	Cpe (2); ? ? ?	A C D	Daube et al. (1996)
Italy	25/87	Cpa (73) Cpa . Etx (14)	A D	Greco et al. 2005

C. PERFRINGENS IN PIGS



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- *C. perfringens* type A is considered by some researchers as the **main cause of neonatal diarrhea** in piglets (Chan et al. 2012)

- The disease is described as a non-hemorrhagic mucoid diarrhea and is characterized by mucosal necrosis and villus atrophy, without attachment and invasion by the microorganism (Songer & Uzal, 2005).

- However, **the pathogenesis of this bacterium in swine remains unclear**, leading to some difficulties in the diagnosis and also making it impossible to determine its true prevalence.

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Country	No of samples/No. of isolates	Genotype (no.)	Type	Reference
USA	333/299	Cpa (225)	A	Baker al. (2010)
Netherland	51/37	Cpa (27) Cpa+Cpb (14)	A C	Klaasan et al. (1999)
Sweden	51/27	Cpa (7) Cpa+Cpb2 (17)	A	Klaasan et al. (1999)
Brazil	90/53	Cpa (53)	A	Ferreira et al. (2012)
Canada	354/225	Cpa (185) Cpa +Cpb2 (40)	A	Chan et al. (2012)

- these recent results suggest that beta - 2 toxin is not important for C. perfringens diarrhea in piglets and also that the use of cpb2 as a virulence factor marker is not appropriate for diagnosis.

- the diagnosis of CPA infection in swine **is challenging**, most authors agreeing that the clinical signs of clostridial diarrhea are similar to those of several other enteric diseases;

- it is also still **not possible to differentiate between pathogenic *C. perfringens* type A and *C. perfringens* type A** that is part of the microbiota.

Clostridium perfringens in poultry

- Necrotic enteritis (NE) in broiler chickens is caused by *C. perfringens* type A and, more uncommonly, by *C. perfringens* type C.

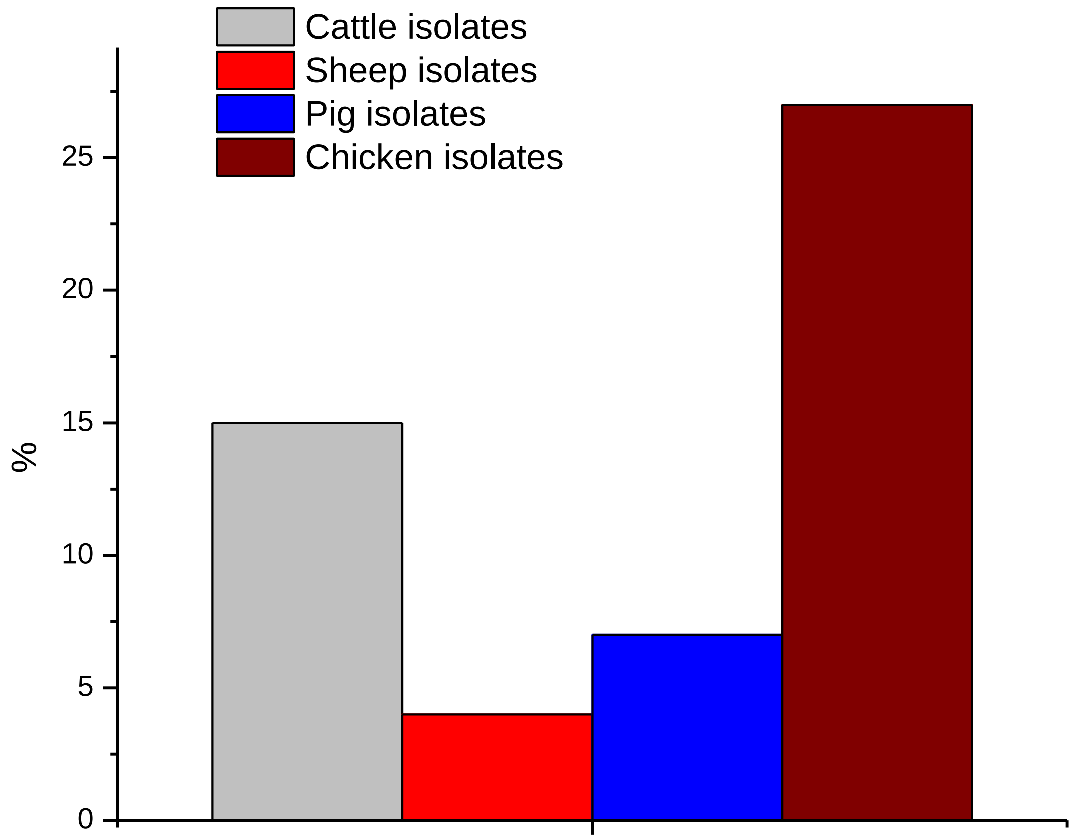
- NE is one of the most common infectious diseases in poultry, resulting in an estimated annual economic loss of more than $2 billion, largely related to impaired growth performance (Cooper et al., 2013).

- *C. perfringens* is found indeed in eggshell fragments, chicken fluff and paper pads in the hatchery, and also was recovered from broiler carcasses after chilling (Craven et al., 2001, Filip et al., 2017).

- It is also shown that intestinal droppings of wild birds contain high numbers of *C. perfringens* and that free-living birds can suffer from necrotic enteritis (Asaoka et al., 2003; Filip et al., 2017).

Country	No. farms/ isolates	Type (%)	Reference
Denmark	25 / 279	A (100%)	Engstrom et al., 2003;
Sweden	10 / 53	A (100%)	Nauerby et al., 2003
Egypt	17/125	A (100%)	Osman et al. 2012
Germany	86/134	A (88.7%) D (7.3%) C (1.6%) E (2.4)	Gad et al. 2012
Czech Rep.	23 / 112	A (100%)	Svobodova et al. 2007
Belgium	31/63	A (100%)	Gholamiandekhordiet al. 2006
Finland	?/118	A (*cpe*-negative)	Heikinheimo and Korkeala 2005.

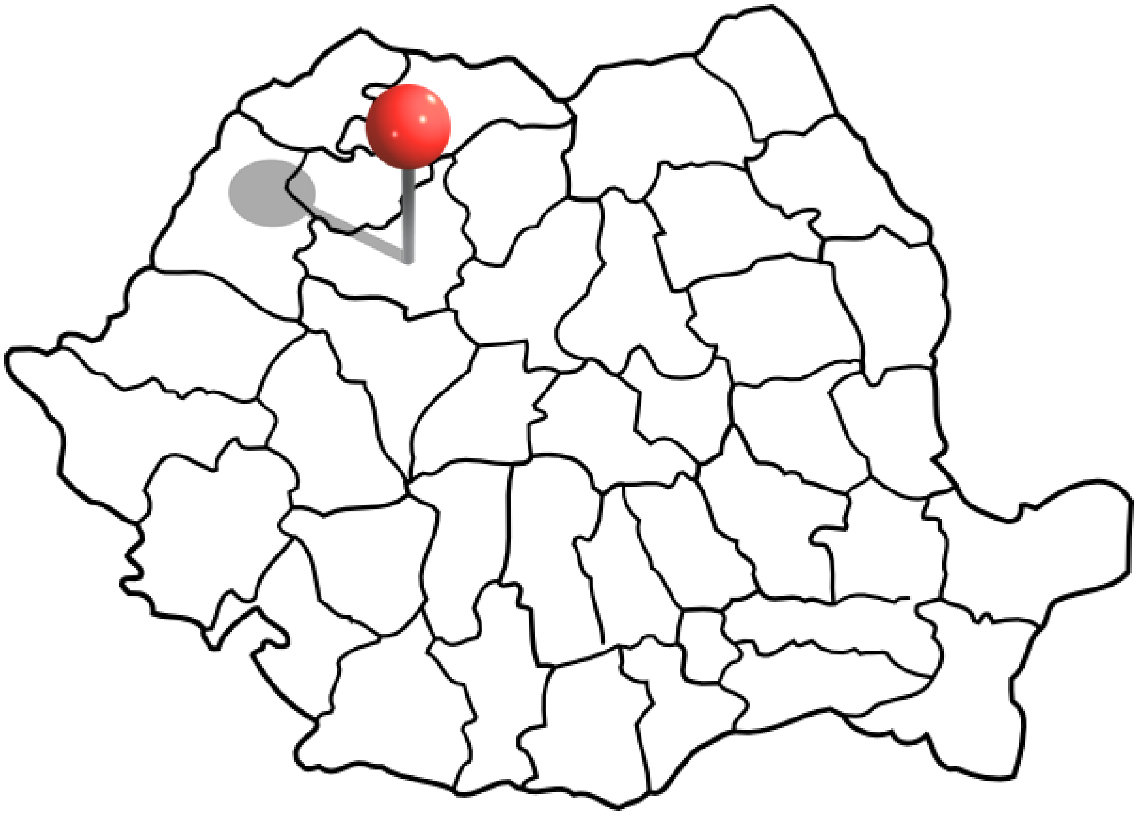
PUBLIC HEALTH RISK



Representation of cpe positive samples in animal isolates from the studies mentioned

CURRENT SITUATION IN ROMANIA

Unpublished data: no. of samples/ positive *Clostridium perfingens* samples 60/54 bovine stool samples, 23/13 ovine stool samples, 76/34 swine samples 23/11 chicken stool samples. Biotype ?



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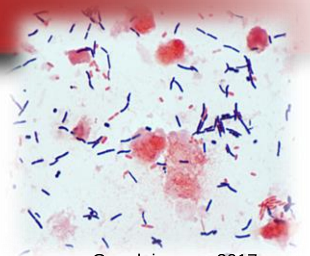
CLOSTRIDIUM DIFFICILE – the risk associated with animals



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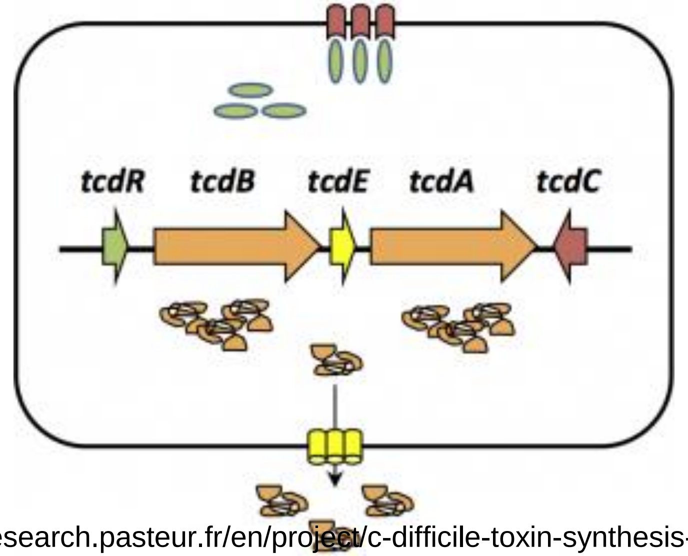
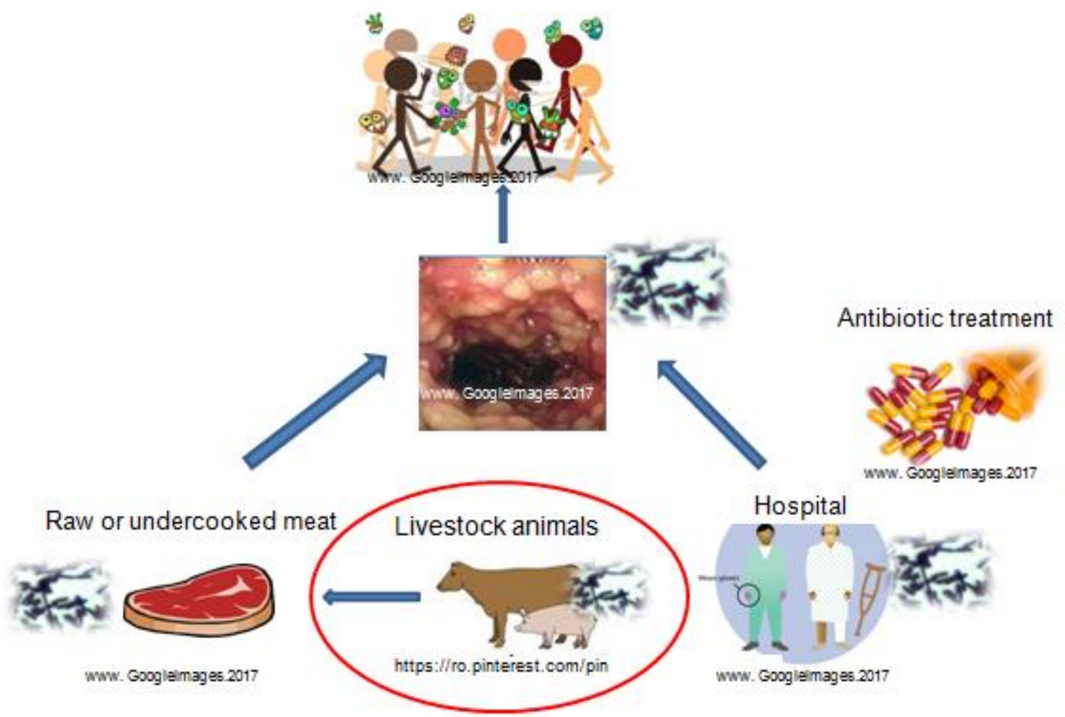
- Anaerobic sporogenic bacterium, recognized as the **major pathogen in healthcare associated intestinal infections in humans** and also as an important animal pathogen (Janezic et al., 2014).
- All virulent strains of *CD* produce two toxin proteins (TcdA and TcdB). The genes encoding both toxins are located within a 19.6 kb region of the chromosome (PaLoc) in addition with three accessory gene (*tcdR*, *tcdC* and *tcdE*).
- Another toxin, binary toxin, also has been described, but its role in disease is not fully understood. (https://research.pasteur.fr/en/project/c-difficile-toxin-synthesis-and-secretion/)



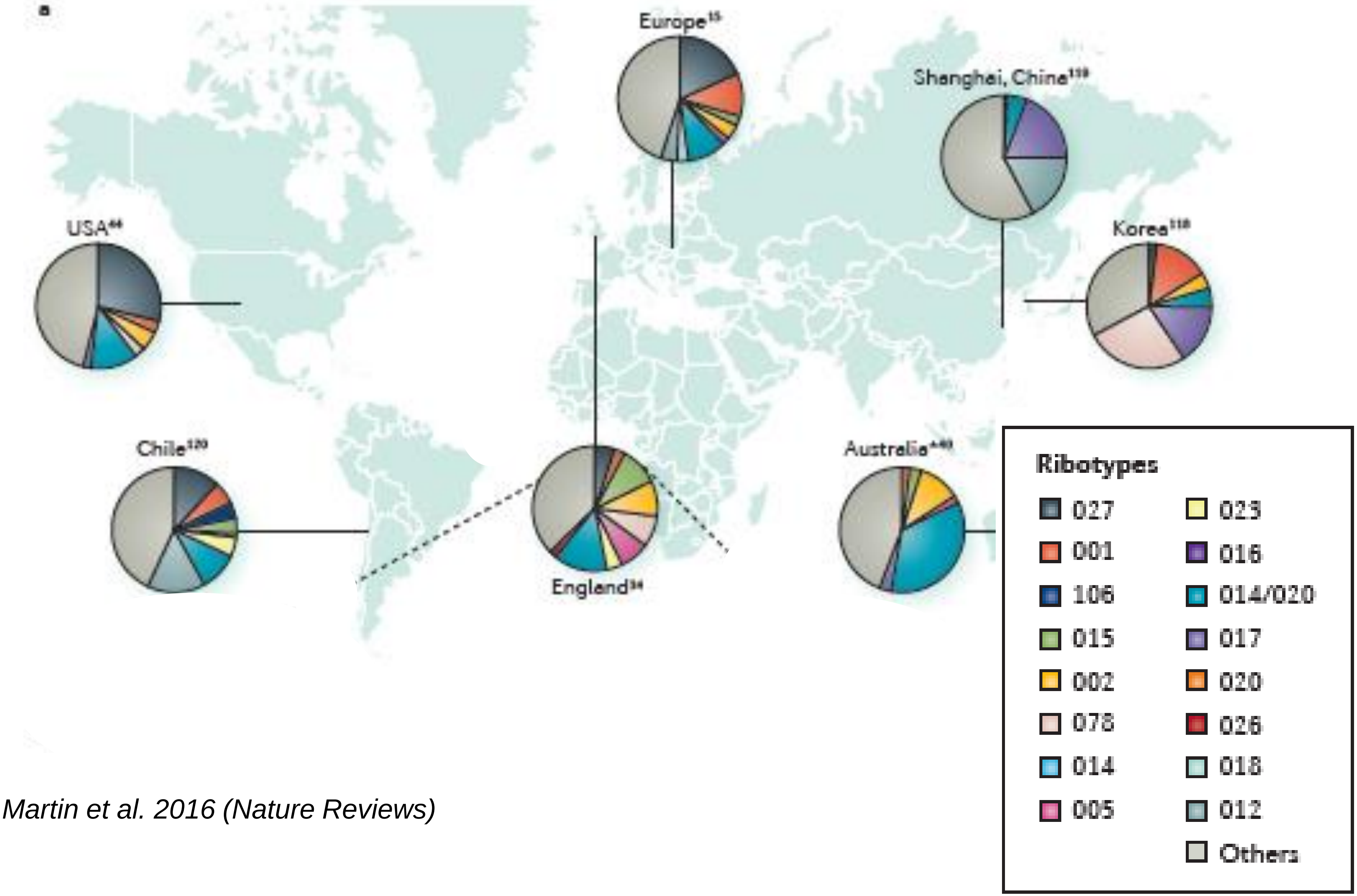
https://research.pasteur.fr/en/project/c-difficile-toxin-synthesis-and-secretion/

➢Two ribotypes associated with outbreaks of severe disease in humans (**017 and 027**) have been found in animals also (Pirs et al., 2008).

➢Modes of transmission between animal and human reservoirs could include retail meat, dog food and contact with the hospital environment (Pirs et al., 2008).



Global epidemiology of common *Clostridium difficile* ribotypes



Martin et al. 2016 (Nature Reviews)

- Although the finding of overlapping PCR ribotypes in animals and humans has stimulated research in this field, the **question of whether zoonotic transmission occurs has not been answered**.

- Circumstantial evidence that *C. difficile* strains from animals were infecting humans (or vice versa) has been reported several times in recent years. These studies have taken animal and human isolates and typed them by molecular methods (Hensgen et al., 2012).

- Whether C. difficile strains in humans and animals are really identical should be determined by, for example, **whole genome sequencing**. The similarities seen in strains of human patients and different animal species do not automatically imply that interspecies transmission occurs (Hensgen et al., 2012).

- In Europe, there is a "hypervirulent" strain of C. difficile, ribotype (RT) 027 (NAP1/BI), which is fluoroquinolone resistant (Loo et al., 2005).

- In addition, RT 078, a similarly virulent predominantly animal strain, is increasingly responsible for human infection in Europe (Goorhuis et al., 2008);

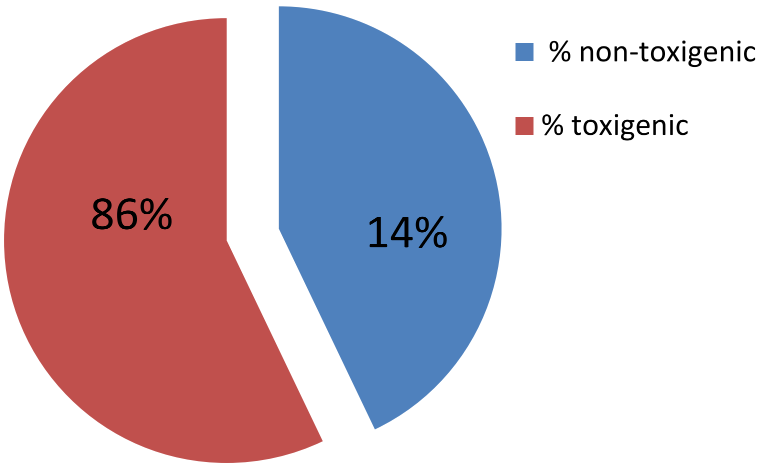
EPIDEMIOLOGY OF C. DIFFICILE IN CATTLE (2012 – 2016)

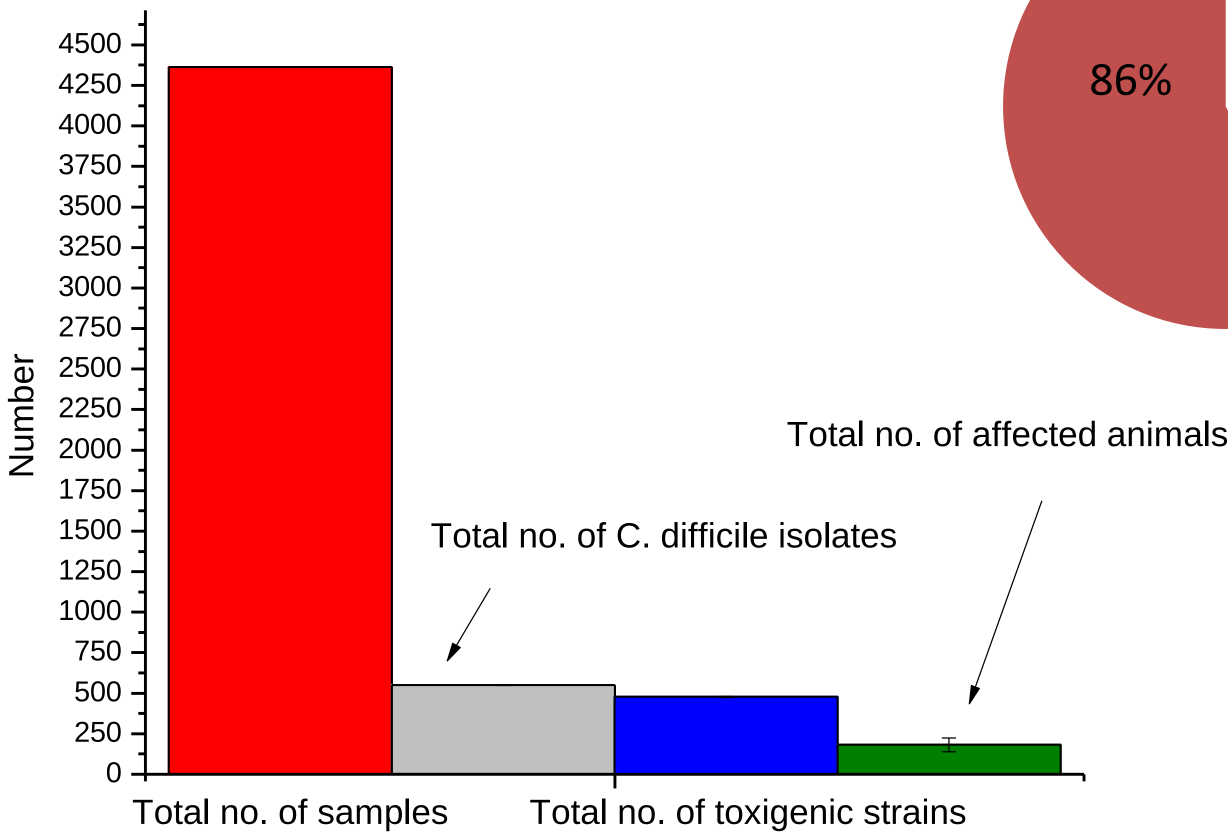
Country	Samples/Isolates	Affected animals	Ribotype (%)	Reference
Germany	999/177	177	**033 (57)** 078 (17) 045 (9)	Schneeberg et al. (2013)
Belgium	101/10	0	**078 (28.6)** 014 (7.1)	Rodriguez et al. (2013)
	202/18	0	**078 (16.6)** 015 (7.1) 002 (7.1) 014 (7.1)	Rodriguez et al. (2012)
Switzerland	110/7	0	**033 (14.2)** 003 (14.2) 066 (14.2) 070 (14.2) 137 (14.2)	Romano et al. (2012)
Slovenia	1051/103	0	**033(?) – predominant** 071(?) 023(?)	Bandelj et al. (2017)

EPIDEMIOLOGY OF C. DIFFICILE IN CATTLE (2012 – 2016)

Country	No of samples/No. of isolates	Affected animals	Ribotype (%)	Reference
Netherland	205/7	5	**012 (85.7)** 033 (14.3)	Koene et al. (2012)
USA	330/2	0	**027 (50)**	Rodriguez-Palacios et al. (2014)
Canada	539/18	0	**078 (100)**	Costa et al. (2012)
Australia	824/209	0	**127 (50.2)** 033 (19.6) 16 (7.7) 126 (5.7) 3 (1.4) 103 (1.4) 7 (3.3)	Knight et al. (2013)

TOXIGENICITY OF C. DIFFICILE IN CATTLE (2012 – 2016)

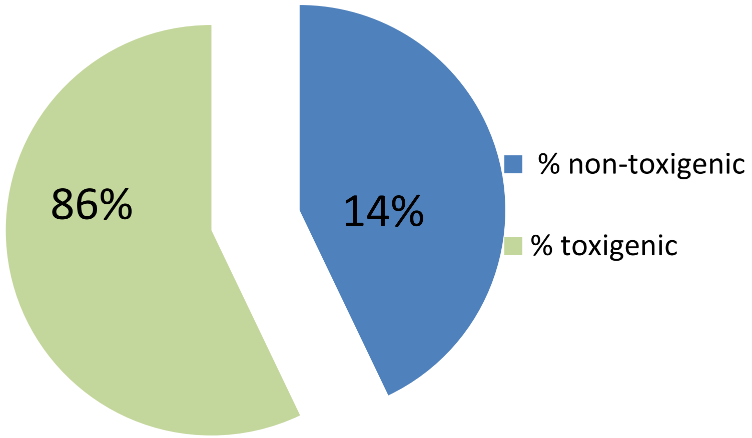


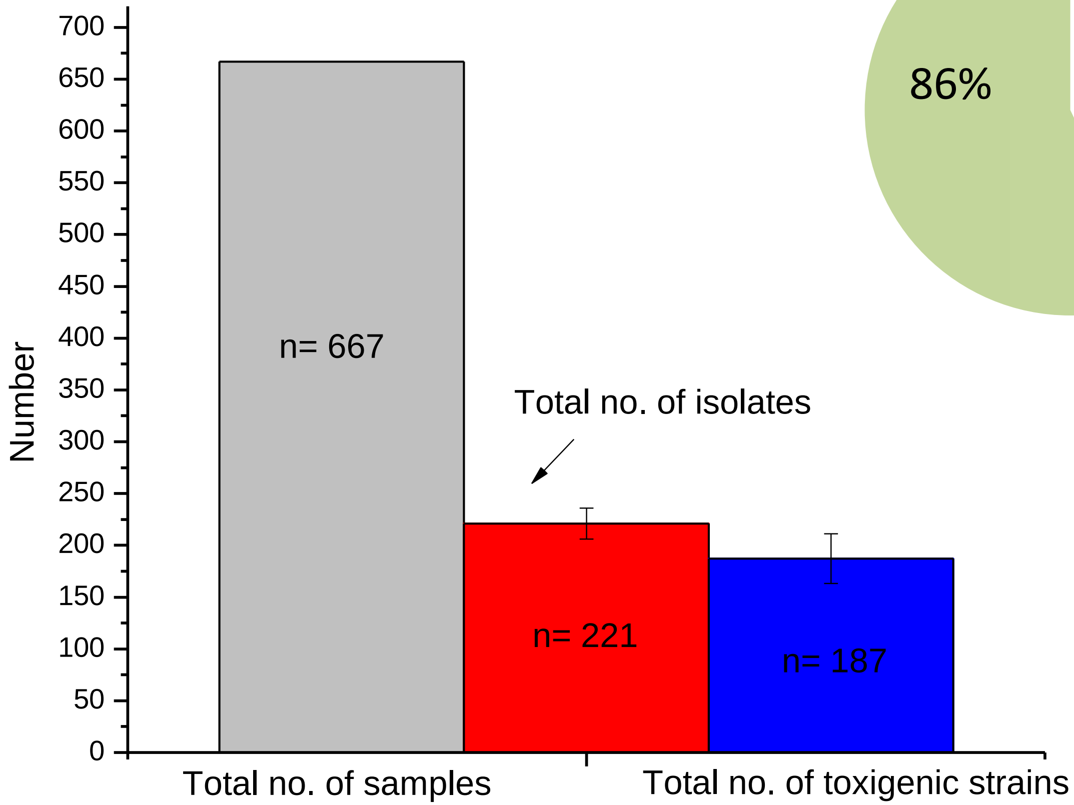


EPIDEMIOLOGY OF C. DIFFICILE IN PIGS (2012 – 2016)

Country	No of samples/No. of isolates	Affected animals	Ribotype (%)	Reference
Sweden	67/45	34	046 (100)	Noren et al. (2014)
Belgium	23/18	0	**078 (66.7)** 002 (16.7)	Rodriquez et al. (2012)
	100/1	0	?	Rodriquez et al. (2013)
Germany	201/147	?	**078 (55)** 126 (20)	Schneeberg et al. (2013)
Netherland	125/9	25	**078 (77.8)** 0.23 (11.1) 0.05 (11.1)	Koene et al. (2012)
USA	150/1	?	?	Rodriguez-Palacios et al. (2014)

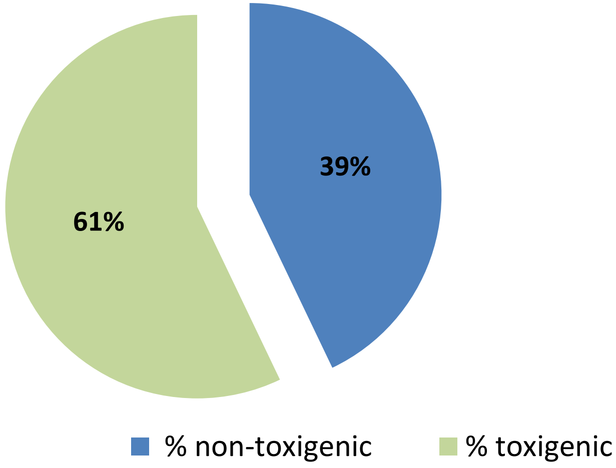
TOXIGENICITY OF C. DIFFICILE IN PIGS (2012 – 2016)





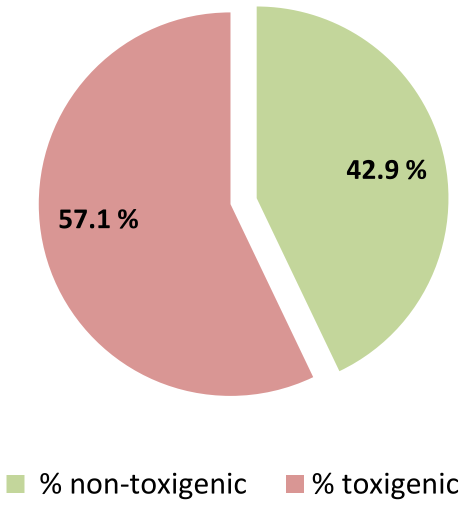
EPIDEMIOLOGY OF C. DIFFICILE IN SHEEP (2012 – 2016)

Country	Samples/Isolates	Affected animals	Ribotype (%)	Reference
Netherland	11/2	11	015 (50) 097 (50)	Koene et al. (2012)
Slovenia	105/6	12	**056 (16.7)** 061 (16.7)	Avbersek et al. (2014)
Australia	371/15	0	**056 (40)** 101 (40)	Knight and Riley (2013)



EPIDEMIOLOGY OF C. DIFFICILE IN POULTRY (2012 – 2016)

Country	No of samples/No. of isolates	Affected animals	Ribotype (%)	Reference
Netherland	121/7	?	014 (28.6) 010 (28.6)	Koene et al. (2012)
USA	340/1	?	?	RodriguezPalacios et al. (2014)



CURRENT SITUATION IN ROMANIA

➢ There are **no published data** regarding C. difficile prevalence in livestock animals;

➢ Little is known about prevailing ribotypes of Clostridium difficile infection in Romania where CDI is not a mandatory notifiable disease.

➢ It is of great concern given that from March 2011 to March 2012, 200 patients were diagnosed with CDI in Bucharest (Rafila et al., 2014).

ONGOING RESEARCH IN THE OUR DEPARTMENT

- project proposal awaiting financing results focusing on : Clostridia isolation from cattle and pigs in slaughterhouses found in Transylvania area – Clostridia isolation from carcasses – Clostridia isolation from retail meat (traditional products also – processed raw); Toxigenicity evaluation, ribotyping, statistical analysis.

http://www.youblisher.com/p/1589980-Borsura-USAMV-Cluj-Napoca/

CONCLUSIONS

- ✓ Although significant progress has been made over the last few decades towards the understanding of clostridia infections in livestock animals, there are still some gaps in the knowledge of the full extent and threat for public health.

- ✓ Further researches must determine the importance of individual toxins in the pathogenesis and virulence of different toxinotypes and strains of these microorganisms.

- ✓ Also, next generation typing techniques must be applied in the future to study the relatedness of strains of human and animal origins so we can evaluate the real threat for public health.

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