CLOSTRIDIUM PERFRINGENS ENTEROTOXIGEN INVOLVED IN HEMORRHAGIC DIARRHEA AT DOGS

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Abstract
Clostridium perfringens is a commensal of the human and animal intestines. The toxigenic strains of this bacterial species are responsible for some enteral diseases in humans and domestic animals. In dogs, hemorrhagic enteritis produced by Clostridium perfringens are sporadic but have a severe progression-restricted progression. In the year 2018, 9 faeces samples were taken from dewormed dogs, vaccinated but with enteritis and associated toxic conditions (vomiting, lethargy, diminished appetite) that started suddenly without any other clinical history. Based on the anamnesis, it excluded the risk of chemical poisoning. Faeces samples were subjected to the microbiological exam. The bacterioscopic examination in Gram stained smears was predominantly Gram positive bacilli with morphology and characteristic disposition for Clostridium sp and no specific morphological phenotypes for spirochetes or protozoa were identified. The samples were incubated at 37° C, under anaerobic conditions, on liquid culture media (bullion VL) and solids (Clostridium agar, SPS agar) and under aerobic conditions on culture media for aerobic bacteria (nutrient broth, blood agar) (E. coli, Salmonella sp., Shigella sp., Campylobacter sp., Yersinia sp., Serpulina sp., Vibrio sp., etc.) which can trigger enteritis in dogs. Following the bacteriological examination, strains of Clostridium sp. Biochemical tests have included the species as Clostridium perfrigens. The clinical progression in the 9 patients was different: 6 dogs responded to antibiotic therapy, recommended on the basis of the antibiotic, and 3 dogs died within 48 hours before a treatment was instituted.

Key words: *Clostridium perfrigens,* enteral diseases, dog

Introduction

Clostridium perfringens is a commensal of the human and animal intestines and it is distributed ubiquitous throughout the environment. It is an anaerobic germ, sporulated, bacillary, Gram positive, encapsulated and unbleached [Carp Carare C., et al, 2014]. The virulence of this bacterium is heavily dependent upon its prolific toxin-producing ability [Jihong Li et al., 2013; Carp Carare C., et al, 2014]. At least 16 different extracellular toxins and enzymes have been identified in five serotypes (A, B, C, D, E) based on 4 major toxins (alpha, beta, epsilon, iota). Certain strains of serotypes A, C, D, E produce an additional enterotoxin (CPE) responsible for the onset of enteric syndrome [Miyamoto K, 2011;Jihong Li et al., 2013]. The toxigenic strains of this bacterial species are responsible for some enteral diseases in humans and domestic animals animals but are also involved in severe diseases such as tissue necrosis, myositis m eningitis, abscesses with various localizations and sometimes septicemia [Jihong Li et al., 2013].

Enterotoxigenic *Clostridium perfringens* contamination of food used in human and animal food leads to multiplication of these in the digestive tube and the production of toxins that triggers a cumulus of symptoms specific to food poisoning [Songer J.G., 1996].

Enterotoxin and α-toxin released from serotype A of the *Clostridium perfringens* species are involved in triggering infectious enteritis in dogs. Same serotype A that is responsible for producing food poisoning in humans [McClane BA, Robertson SL, Li J. 2013]. Being a commensal of the intestinal mucosa, *Clostridium perfringens* type A, can colonize the intestine without triggering an infectious process. Even in this situation, antibiotic treatments can activate the plasmid encoding the enterotoxin that causes a non-alimentary gastrointestinal disorder [Carman RJ. Et al., 1997;Miyamoto K, et al., 2006.*cit.* Jihong Li et al., 2013].

Materials and methods

In 2018, 9 faeces samples were collected from dogs of different ages and breeds, raised in open space. All dogs were deparasized and vaccinated. Enteric syndrome started in 12-24 hours without known causes. Enteritis has evolved with abdominal cramps and hemorrhagic diarrhea, associated with symptoms specific to a toxic condition: vomiting, lethargy, diminished appetite. Based on the anamnesis, the risk of chemical intoxication was avoided.

The samples were incubated at 37°C, under anaerobic conditions, on the usual culture media for anaerobic bacteria (Broth VL, Blood Agar, Sulfite Agar-Polymixine B-Sulfadiazine). In order to isolate some bacterial strains that can trigger enteritis in bacterial dogs (*E. coli, Salmonella sp., Shigella sp., Campylobacter sp., Yersinia sp., Serpulina sp. Vibrio sp.,* etc.), common culture for aerobic bacteria (Nutrient Broth, Blood Agar).

Bacterial strains that have developed on anaerobic media have been biochemically tested using API 20A galleries. Sulfitoreductant capacity was assessed on Agar SPS medium (Sulfite-Polymixine B-Sulfadiazine agar).

The antibiogram was performed by the diffusion method on the SPS Agar medium, under anaerobic conditions

Rezults and discussion

The bloody faeces samples (fig.1) were subjected to the microbiological examination from the suspicion of an intestinal infection. In the direct bacterioscopic examination, Gram-positive bacilli with morphology and characteristic disposition for *Clostridium* sp. predominated (fig.2), spores of *Clostridium sp* (fig.3) and no specific morphological phenotypes for spirochetes or protozoa were identified.

Fig.1. The faeces samples with blood

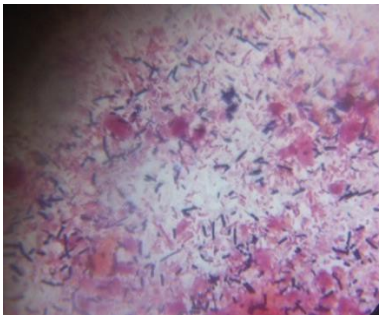

Fig.2. Gram-positive bacilli with morphology and characteristic disposition for *Clostridium sp.*-direct bacterioscopic examination,MO, x1000

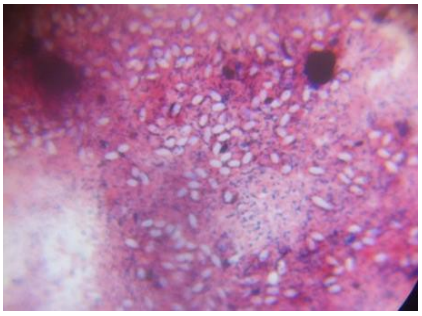

Fig.3. Spores of *Clostridium sp.*-direct bacterioscopic examination, MO.x1000

The cultural features of the isolated strains in liquid anaerobic culture media where intense turbidity (fig.4) and foam on the surface of the broth and rancid smell. On solid media, the cultural characters were different depending on the culture medium on which the bacteria had developed. On the blood agar, S-type colonies were formed with opaque gray, beta-hemolytic center, and Agar

SPS (Sulfite-Polymixina B-Sulfadiazine) developed dark-colored lenticular-discoidal colonies) with gas production (fig.5).

After the smears were made from isolated cultures on these mediums and Gram stained, the Gram positive, short and thick bacilli (4-8 / 1.5 um) were observed at the optical microscope, with rounded heads, sometimes grouped by diplo or grouped by two-joints (fig.6).

Fig. 4 *Clostridium perfringens* - Broth VL medium

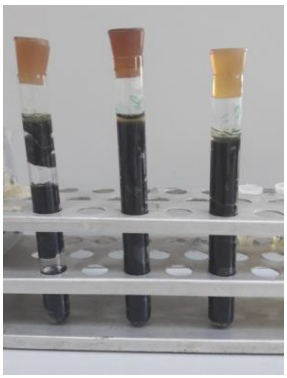

Fig. 5 *Clostridium perfringens* Agar SPS Medium

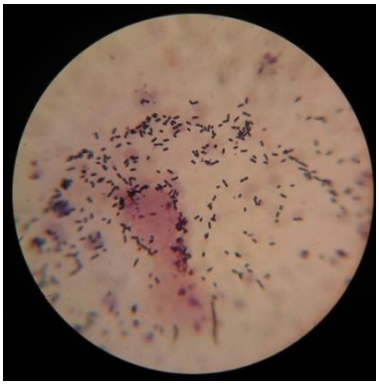

Fig. 6. *Clostridium perfringens*, Gram stain, MO,x 1000

Isolated strains were biochemically tested using the Api 20A galleries, which are 88% probable confirmed as belonging to the *Clostridium perfringens* species (fig.7)

Fig. 7 *Clostridium perfringens,* miniAPI 20A gallery

In order to institute an anti-infectious treatment, antibiotics were performed on all strains of *Clostridium perfringens*. The antibiotics currently used in the digestive antiinfectious therapy, adapted as appropriate: enrofloxacin, marbofloxacin, streptomycin, gentamicin, lincospectin, clindamycin, vancomycin, ampicillin-amoxicillin, oxitetracycline, colistin, norfloxacin, amoxicillin-clavulanic acid, ceftiofur, oxytetracycline, cefaclor.

Fig.8 Antibiograms on *Clostridium perfringens* strains on Agar SPSs -exemplification

The results of the antibiotics varied according to the strain (fig.8, table 1)

The sensitivity profile of the isolated strains was different, with the percentage of sensitivity and resistance varying greatly from one case to another.

Table 1

Antimicrobial susceptibility of *Clostridium perfringens* strains

Nr.	antibiotice											
	ENR	MAR	CIP	LCS	GN	DA	S	VA	AX	AMC	CEC	TE
T1	S	S	S	S	R	S	S	S	S	/	/	/
T2	R	S	/	S	R	S	S	/	S	I	I	/
T3	R	I	R	I	R	R	R	I	I	/	/	/
T4	S	S	/	R	R	S	/	S	/	R	I	R
T5	R	S	/	R	I	I	/	R	/	S	S	/
T 6	R	S	R	R	R	S	R	R	R	R	/	/
T7	R	R	R	R	R	R	/	R	R	R	R	/
T 8	S	S	/	S	/	S	R	/	S	S	I	R
T9	R	S	/	S	/	R	/	R	I	R	R	R

From the analysis of the data obtained, it was found that the most effective antibiotics tested in vitro were: marbofloxacin (77%) and clindamycin (55.55%). *Clostridium perfringens* showed a high degree of antimicrobial resistance to oxitetracycline (100%), getamycin (85.71%), ciprofloxacin (75%), enrofloxacin (66.66%), streptomycin (60%), vancomycin amoxicillin + clavulanic acid (57.11%) (fig.8).

The clinical progression in the 9 patients was different: 6 dogs responded to antibiotic therapy, recommended on the basis of the antibiotic, and 3 dogs died within 48 hours before a treatment was instituted.

Spores of *Clostridium prfringens*, being ubiquitous, can contaminate food used in human and animal food. From anamnesis it turned out that these dogs included in feed included the grains

of meat bought from commerce as raw or boiled meat, discreetly placed in the accommodation where they stayed during the day at the specific summer temperature. In these cases, there was suspected *Clostridium perfringens* food allergy produced by released enterotoxin, most likely serotype A [Jihong Li et al., 2013]. Consumption of food contaminated with *Clostridium perfringens* and the existence of favorable factors (hyperproteic diet, moldy foods, tachyphagia, etc.) leading to diminished intestinal peristalsis and pH changes in the digestive tract allow these microorganisms to multiply and release large quantities of enterotoxin , responsible for the symptoms of these conditions.

Conclusions

1. *Clostridium perfringens* has been incriminated in the appearance of food poisoning in dogs of different ages and breeds.
2. The cause of toxic infections was the consumption of contaminated meat and meat products.
3. The favorable causes were the hyperproteic regime, the *ad libitum* food administration and the temperatures that allowed the germs to multiply in food.

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