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OBSERVATIONS ON CLOSTRIDIUM SPIROFORME AND RABBIT ENTEROTOXAEMIA

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Introduction

Until the recent work of Borriello and Carman[1] the aetiology of both spontaneous and antibiotic-associated diarrhoea and colitis in rabbits was unknown. The first good indication that a bacterium might be involved in the disease process was the detection of a toxin in the caecal contents of diseased animals which could be neutralized by *Clostridium perfringens* Type E anti-toxin (Katz et al 1978). However, *C. perfringens* were rarely present in diseased animals and when found were Type A. This dilema was resolved when it was shown that there was a striking association between carriage of *C. spiroforme* and disease, and further that *C. spiroforme* was the source of the toxin that could be neutralized by *C. perfringens* Type E anti-toxin (Carman and Borriello, 1982 and Borriello and Carman 1983)

C. spiroforme and disease in rabbits

The evidence that *C. spiroforme* causes the disease seen in rabbits is as follows: –

1) *C. spiroforme* is always present in high numbers in diseased rabbits but not found in totally asymptomatic animals (Borriello and Carman 1983).

2) *C. spiroforme* produces the toxin that is found in diseased animals (Borriello and Carman 1983).

3) Intracaecal administration of *C. spiroforme* results in the in-vivo production of this toxin (Carman et al 1984).

More recent work of ours which we intend to present in detail has shown that: –

4) Oral administration of *C. spiroforme* to antibiotic treated adult rabbits results in disease; whereas if these animals are kept free of exposure to the organism they survive.

5) Oral administration of *C. spiroforme* to weaning rabbits results in disease; whereas animals kept free of exposure to the organism survive.

Points 4 and 5 are also evidence for the disease being an infection, as opposed to overgrowth of undetectable levels of the organism that may be resident in the gut, as in both cases exposure of the susceptible animal to *C. spiroforme* was necessary to induce disease. In the absence of exposure to *C. spiroforme* the animals survived.

Host Spectrum

In addition to rabbits, administration of *C. spiroforme* to antibiotic pre-treated adult animals will cause disease in guinea pigs, hamsters, rats, mice

and cottontails (Carman and Borriello, 1984; Borriello and Carman 1984).

Characteristics and detection of *C. spiroforme*

C. spiroforme is a Gram positive sporeforming anaerobic bacillus that can exist in a helically coiled state (Kaneuchi et al. 1979). Most recently we have shown by scanning electron microscopy that both helically coiled forms and C-shaped individual cells are apparent and that in many cases the individual cells coiled on to themselves to form a 'twist'. In addition our observations showed that the helically coiled forms of the organism were in fact chains of individual semi-circular cells joined end-to-end.

Direct Gram stain screening of material from diseased animals can be a useful aid in the detection of *C. spiroforme*, as the Gram film should be characterized by the presence of many semi-circular bacteria (Carman and Borriello, 1983). However, although suggestive of the disease it is not a reliable diagnostic criterion. Isolation of the organism is aided by the fact that one can use simple spore selection techniques which makes isolation relatively simple (Borriello and Carman 1983). A selective medium has yet to be developed although initial studies based on the addition of neomycin, rifampicin and bacitracin to agar media have proved encouraging (Carman and Borriello 1983). An indirect method of inferring the presence of *C. spiroforme* is to look for its toxin (see below). It can be seen that if toxin is present in faecal material *C. spiroforme* is also present (Table 1).

Detection and characteristics of the toxin

Cell free filtrates of caecal material or culture supernatants that contain *C. spiroforme* toxin are lethal for mice when injected intraperitoneally. Hind-limb paralysis usually precedes death. Intradermal challenge of depilated guinea pigs

produces a characteristic necrotic lesion. The toxin will also induce fluid accumulation in the infant mouse or ligated rabbit ileal loop. In all cases the effects can be neutralized by the cross-reacting *C. perfringens* Type E anti-toxin. An alternative to animal detection systems is the use of tissue culture. The toxin is cytotoxic to VERO, MRC5 and HeLa cells and can be specificaly neutralized by *C. perfringens* Type E anti-toxin.

The toxin is heat labile (56^{0}C for 30 minutes), and may exist as a pro-toxin, as *C. spiroforme* toxin produced *in vitro* is biologically active only after pre-treatment with trypsin.

Conclusions

The above observations on the biological activity of the toxin produced by *C. spiroforme* and the development of an isolated rabbit model of the disease should greatly increase our understanding of the pathogenesis and control of rabbit enterotoxaemia.

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Table 1

Concordance of *C. spiroforme* and a toxin neutralized by *C. perfringens* Type E antitoxin

	C. spiroforme +ve	*C. spiroforme* -ve
Toxin +ve	31	0
Toxin -ve	2	70

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Summary

Clostridium spiroforme and its associated toxin have been shown to be the cause of both natural and antibiotic induced enterotoxaemia in rabbits. In adult animals both pre-treatment with antibiotics and exposure to *C. spiroforme* are required to induce disease, neither alone being sufficient. In weanling rabbits exposure to organism alone is sufficient. The toxin has been shown to be heat labile, cytotoxic to tissue culture cells, dermonecrotic in guinea pigs, lethal to mice upon intraperitoneal injection and able to produce fluid accumulation in ligated rabbit ileal loops and suckling mice.

Italian Summary

Enterocolite é una grave malattia gastrointestinale dei conigli. La malattia può associata alla somministrazione di antibiotici ed il ruolo eziologico del *Clostridium spiroforme* e della sua tossina é stato dimostrato negli ultimi anni da Borriello e Carman.

La tossina é stato presentato come citotossico ed enterotossico. Le attività venivano neutralizzate dall'antitossina *Clostridium perfringens* Tipo E.