

## NOTE

### THE OCCURRENCE OF VEGETATIVE CELLS OF CLOSTRIDIUM PERFRINGENS IN SOIL

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Received for publication June 30, 1949

A considerable difference of opinion exists as to the form in which the pathogenic clostridia, and particularly *Clostridium perfringens*, exist in the soil. Some authors consider that the soil is a natural habitat of this species. Others believe that *C. perfringens* leads a saprophytic life in the intestinal contents of man and other animals, that the organisms are excreted with feces, and that only spores resulting from fecal contamination are to be found in the soil.

In order to obtain information on this point the relative numbers of heat-susceptible and heat-resistant forms were determined in eight samples of soil that had not been exposed to known fecal contamination for some years. Duplicate portions of each soil sample were diluted  $10^1$  in sterile M/60 phosphate buffer (pH 7.0). One of each pair of  $10^1$  dilutions was heated for 20 minutes at 78 C. Serial dilutions in phosphate buffer of  $10^2$ ,  $10^3$ ,  $10^4$ , and  $10^5$  were then made from the unheated and heated suspensions. From each dilution 10 tubes of chopped meat medium (pH 7.4) containing 1 per cent glucose were inoculated with 0.5 ml and were incubated for 8 to 12 hours at 37 C. From each chopped meat tube a tube of whole milk medium was inoculated, and the milk tubes were incubated for 12 to 18 hours. From each milk tube showing production of acid and gas, a blood agar plate was inoculated. After anaerobic incubation of these plates, colonies resembling those of *C. perfringens* were transferred to semisolid medium for the determination of motility and morphological characteristics. The ability of a number of the strains to ferment glucose, lactose, sucrose, and salicin was determined. The "most probable number" of organisms per gram of soil (on a dry weight basis) for each sample was calculated by the method of Halvorson and Ziegler (*Quantitative Bacteriology*, Burgess Publishing Co., Minneapolis, 1938), with 10 tubes for each dilution as is indicated above. The results are shown in table 1.

It should be pointed out that the numbers of organisms presented in this table are based on actual isolations. The true numbers of *C. perfringens* in the various samples may be larger, because of inadequacies of the technique used for isolation, but they are quite unlikely to be smaller. Comparative data on heat-susceptible forms of *C. perfringens* were not obtainable for one sample of soil because it contained such large numbers of an anaerobic micrococcus, which produced "stormy fermentation" of milk, that *C. perfringens* could not be isolated on a quantitative basis.

The data in table 1 indicate that large numbers of *C. perfringens* can exist in the soil in heat-susceptible form. Because of the relatively high heat resistance

TABLE 1  
*Numbers of C. perfringens per gram (dry weight) of soil*

ORIGIN OF SOIL SAMPLE	pH OF SOIL	HEAT-RESIS- TANT	TOTAL
Clayey loam from uncultivated field.....	5.4	790	1,090
Black loam from creek bank.....	5.3	5,940	56,700
Sandy garden loam.....	6.0	703	1,730
Clayey garden soil.....	5.7	73	110
Muck from swamp.....	4.5	464	1,530
Sandy soil and decaying vegetation.....	6.0	200	1,150
Soil underlying turf.....	6.3	70	1,200

of *C. perfringens* spores reported by Weinberg, Nativelle, and Prévot (*Les Microbes anaerobies*, Masson et Cie., Paris, 1937), it appears that the heat-susceptible forms are probably vegetative cells. It follows that multiplication of this species takes place in the soil, and, consequently, that soil is a natural habitat of this species and that it is not necessary to postulate fecal contamination to account for its presence in soil.