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Confocal and SEM Studies of Protist Parasites on Fresh Produce

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Human waterborne parasitic protists such as *Cryptosporidium*, *Blastocystis, Giardia* and microsporidia contaminate a variety of fresh produce worldwide. Foodborne outbreaks involving these organisms impacting hundreds of people have been linked to consumption of fresh salads, fruits, and unpasteurized juices. Fresh fruits and leafy vegetables might have been contaminated from irrigation and wash waters and/or contact with the soil or processing equipment. We have studied the persistence and viability of various protist parasites on fruits and vegetables experimentally contaminated with water suspensions of these pathogens. Apples contaminated with *Cryptosporidium* oocysts and stored in the cold for 4-6 weeks and leafy vegetables similarly contaminated were observed periodically 5-10 days using confocal laser scanning microscopy (CLSM) and low temperature SEM (LT-SEM). Microscopic observations found some oocysts were discovered deep in natural crevices in the apple peel (Figure 1A) [1], whereas others were attached to the smooth surface of the peel and on leaf surfaces (Figure 1B) [1, 2]. Using CLSM and 3D reconstruction studies we discovered that *Cryptosporidium* oocysts can infiltrate through the stomatal openings (plant epidermal pores responsible for gaseous exchange) into the leaves, and persist internally within the leaf (Figures 1C&1D) [2]. This is the first evidence of any protozoan parasite capable of infecting humans or animals to be shown sequestered within fresh vegetable produce. A new immunoflorescent reagent has been developed and validated to specifically identify *Blastocystis* spp. in complex mixtures of protozoan pathogens and can be potentially applicable to screening of fresh produce using CLSM and immunefluorescence microscopy techniques (Figure 1E) [3]. Standard surveys of fresh produce use elution techniques to detect potential contamination with human enteric pathogens. A rapid adhesive tape technique was developed to sample the produce surface for contamination. Our LT-SEM and CLSM studies of apple, cucumber, tomato and peach contaminated with protist pathogens discovered that produce with smooth surfaces such as apple, spinach, cucumber and tomato are readily identified, however, the epidermal hairs found on peach prevented many of the pathogens from being detected using adhesive tape (Figure 1F) [4]. Our experiments prove that under experimental conditions waterborne protist pathogens can adhere, persist and remain infectious on produce meant to be eaten raw. Furthermore, protozoan pathogens can become internalized into fresh produce that makes washing and sanitizing inefficient. Extension of these findings to the possibility and likelihood that this occurs under natural conditions raise concerns regarding food safety. Several USDA-ARS laboratories are conducting experiments to determine the best method for detection and removal of these pathogens from fresh produce.

References

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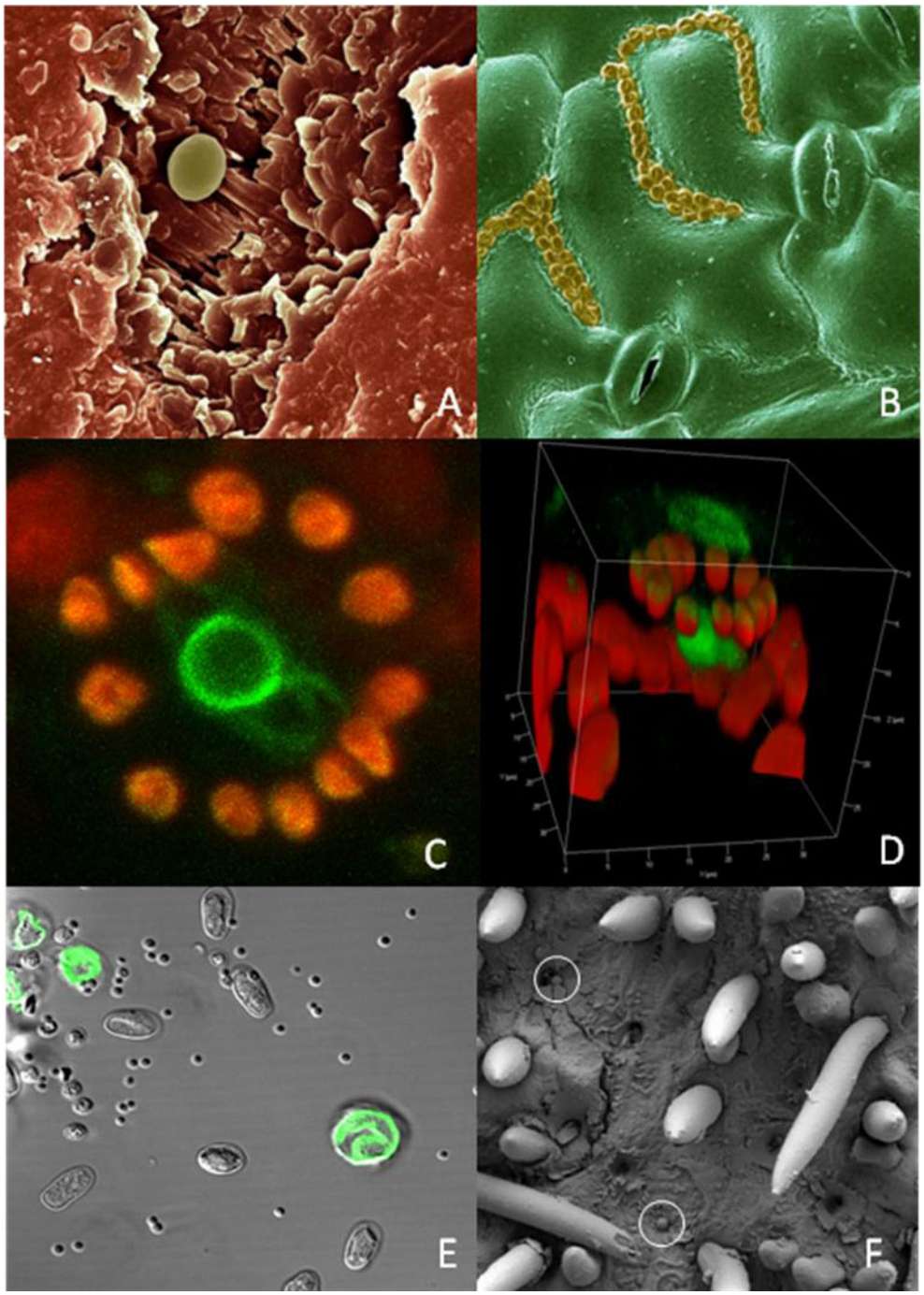

Figure 1. A. Colorized LT- SEM image of *Cryptosporidium* oocyst (green) in crevice of apple fruit peel; B. Colorized *Cryptosporidium* oocyst (yellow) on surface of spinach leaf; C. CLSM Z-Stack image of *Cryptosporidium* oocyst (green) inside stomata of a spinach leaf with autofluorescent choloplasts (red); D. CLSM 3D reconstruction of optical sections through a spinach stomata showing the *Cryptosporidium* oocyst (green) inside the stomata cavity; E. CLSM Blastocystis spp. fluorescently labeled (green) amongst other non labeled mixed protists; F. *LT- SEM Cryptosporidium* oocyst (circles) on surface of peach fruit peel amongst peach fruit trichome hairs.

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