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Letter to the Editor

LEGIONELLA PNEUMOPHILA IN A HUMAN TROPHOBLAST CELL LINE

Dear Editor:

Growth impairment in a human trophoblast cell line (JEG-3, ATCC HTB 36) led to the isolation of *Legionella pneumophila*. A contamination of cell cultures with this pathogen has not been reported so far.

The supernatant medium of insufficiently growing JEG-3 cells remained clear without abrupt pH change. Only light microscopy revealed a vacuolization and intravacuolar structures. But these structures gave no distinct reaction to gram stain and could not be eradicated by penicillin, gentamicin and streptomycin. A cultural isolation of bacteria did not succeed on universal agar media.

After all, bacteria with inner and outer membrane were shown electron microscopically (Fig. 1). In degenerated JEG-3 cells of the same specimen the intravacuolar bacteria had a more electron scattering periplasmic space (Fig. 2). which could explain the slightly grampositive or intermediate gram stain reaction. Finally, *Legionella pneumophila* was identified on yeast charcoal agar after prolonged incubation (1).

In further JEG-3 subcultures, methods for rapid diagnosis of *Legionella pneumophila* were tested. Without need for cultural isolation of the bacteria, direct immunofluorescence with polyvalent antibodies (Mardy Diagnostics, Inc.) allowed the identification of intracellular *Legionella pneumophila*. Also hybridization with a Legionella-specific DNA probe (Gen-Probe, Inc.) detected bacterial rRNA in sonicated JEG-3 cultures.

Penetration and replication of *Legionella pneumophila* have been reported for experimentally infected human (3,5,7) and animal (4,8,9) cells other than JEG-3 cells. Like in JEG-3 cells, the bacteria multiplied within ribosome-studded vacuoles (2,3) stressing the assumption that the cellular reaction is induced by the bacteria independently from eukaryotic specificity.

The lack of information concerning Legionella contaminations

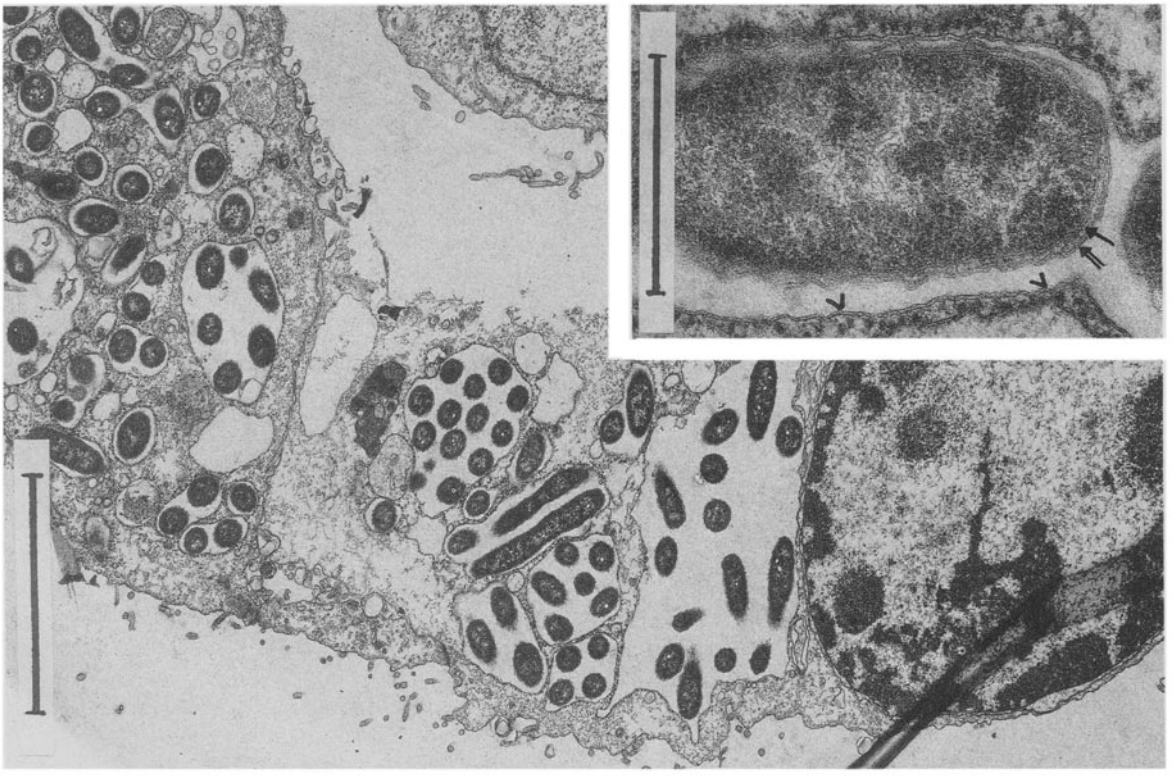

FIG. 1. *Legionella pneumophila* in cytoplasmic vacuoles of Epon embedded JEG-3 cells. *Insert* shows the gramnegative bacterium adjacent to the ribosome-studded vacuole membrane (*arrowheads*). → inner membrane, ⇒ outer membrane. *Bars* represent 5 μm and 500 nm respectively.

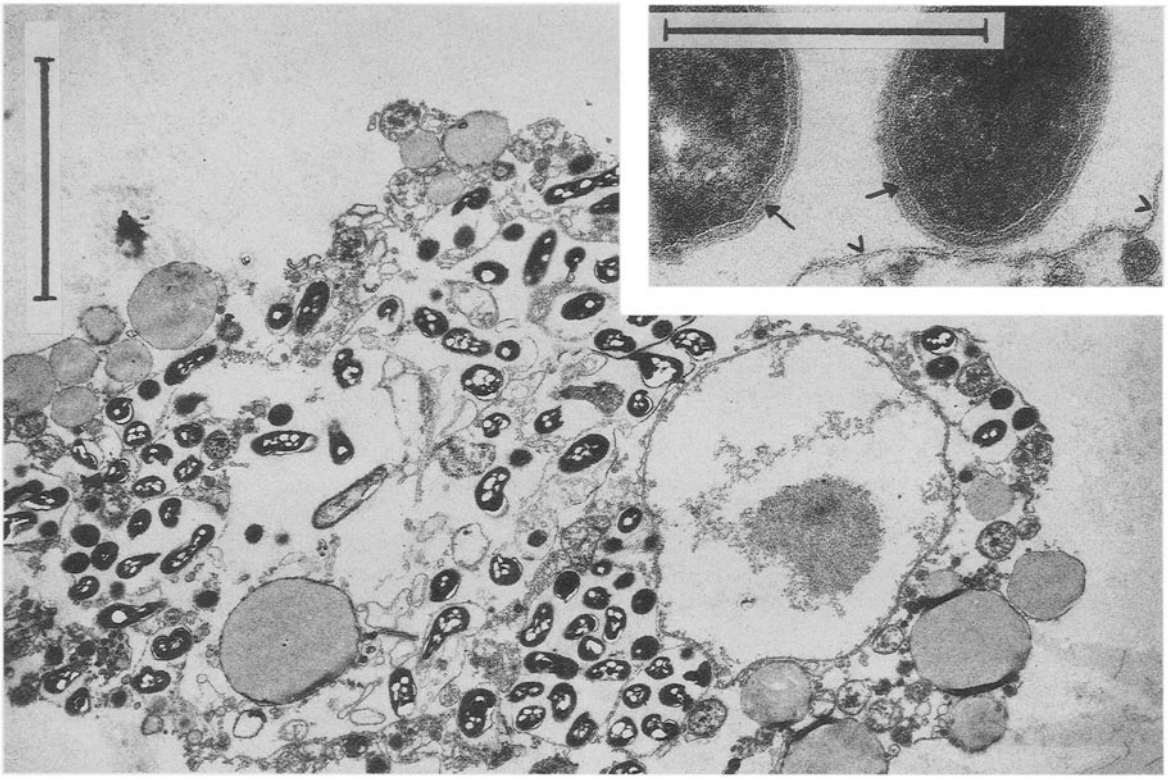

FIG. 2. *Legionella pneumophila* in a destructed JEG-3 cell (found in the same section among intact cells). *Insert* shows part of the ribosome-depleted vacuole membrane (*arrowheads*) and bacteria with a dense electron scattering periplasmic space →. *Bars* represent 5 μm and 500 nm respectively.

might simply be due to their infrequency or to the more elaborate microbiological requirements. Most appropriate sources of contaminations would be water pipes, air conditioning systems and even distilled water where osmotic resistant *Legionella pneumophila* (6) might be selected from more sensitive species.

DNA-probe and immunofluorescence are recommended for screening.

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