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Brief Communication

UREAPLASMA ISOLATED FROM THE RESPIRATORY TRACT OF MINK

On examination of the lungs of 21 mink kits originating from eight farms, ureaplasma-like organisms were found in three animals from two farms. Two of the isolates were obtained from three-week-old scanblack kits in a farm where sudden deaths had occurred in some litters. The lungs of the dead kits were congested, edematous and firm. The histologic picture was dominated by hyperemia and edema. The interalveolar septa were thickened, with a moderately increased number of cells. Some mononuclear cells and leucocytes were found in the alveoli. The organisms were isolated from two out of five animals examined. No bacteria could be demonstrated. The third ureaplasma-like organism was isolated from the apparently normal lungs of a two-month-old kit from another farm.

The isolates (U58 and U59) from the farm where a disease complex with pulmonary involvement had been observed were examined with respect to the correctness of referring them to the genus Ureaplasma within the class Mollicutes. The medium used was originally developed for propagation of Mycoplasma suipneumoniae (*Friis* 1975). It was enriched with 20 % horse serum and with urea, 0.5 mg/ml, and $MgSO_4$, 0.2 mg/ml. The pH was adjusted to 6.75.

Both strains were passed through a membrane filter (Gelman, TCM 0.45 μm) and cloned once on solid medium. Hereafter they were passaged five times in liquid medium without antibiotics. The last passage was examined by interference-contrast microscopy, and by light microscopy of smears stained after the method of Gram and with carbol-methylene blue. No bacteria could be demonstrated. The filtration-cloning process was repeated twice, and two clones were finally isolated from each strain and examined further.

When cultivated in liquid medium at 37 C all four clones produced a reddish-blue colorshift of the phenol-red indicator, signifying a splitting of urea. On solid medium in an atmosphere of air + 5-10 % CO_2, colonies $\leq$ 0.1 mm developed after 24-28 h of incubation. Small colonies appeared granular, while the bigger ones had a dark central nipple surrounded by a lighter zone. All

the colonies were stained brown with the conventional solution of $MnCl_2$ + urea.

As judged by the color changes during growth in liquid media enriched with 0.1 % urea, glucose or arginine, all four clones split urea, but neither glucose nor arginine. No growth appeared to take place in liquid medium without serum. In the conventional SPS and digitonin tests performed on solid medium all four clones showed zones of inhibition of approx. 5 mm around the SPS discs and of approx. 10 mm around the digitonin discs.

All four clones were found to pass membrane filters with pore size 0.6 μm, also such with pore size 0.45 μm, although with great loss. Pore size 0.3 μm was passed by just one clone and 0.2 μm by none. With a prefiltration growth end point of 10^{-5}, Strain U59, clone A showed end points of 10^{-3}, 10^{-1} and 0 after filtration through, respectively, 0.6, 0.45 and 0.3 μm membranes.

All four clones were examined by the disc growth inhibition test and the metabolism inhibition test against antiserum* for the type strain of Ureaplasma urealyticum (T960-serotype VIII). No inhibitory effect was noted on any of the clones.

Transmission experiments were performed with a crude isolate from the lung from which Strain U59 was finally isolated. Of a third-passage culture in liquid medium representing dilution 10^{10} of the original tissue, 1 ml, containing $10^{5.7}$ ccu, was inoculated into the nasal cavity of four six-week-old mink kits. Two animals were sacrificed after five days and two after eight days. No clinical signs of disease were observed and no lesions were found at necropsy. Ureaplasmas, while not being reisolated from the lungs of any of the animals, were found in the nasal cavity of one animal.

For electron microscopy, pellets obtained from broth cultures were fixed with glutaraldehyde and osmium tetraoxide. Sections were stained with uranyl acetate and lead citrate. In all four clones pleomorphic organisms surrounded by a triple-layered membrane were observed (Figs. 1, 2 and 3). Fig. 3 shows the special arrangement of ribosomes in parallel rows, which has been observed earlier in ureaplasmas by other workers (*Vinther* 1976). By interference-contrast microscopy of cultures in prefiltered medium (0.2 μm) pleomorphic coccobacillary elements, lying alone or in small clusters, were seen in all four clones.

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* The antiserum was kindly provided by Dr. A. C. Thomsen, the Institute of Medical Microbiology, University of Aarhus, Denmark.

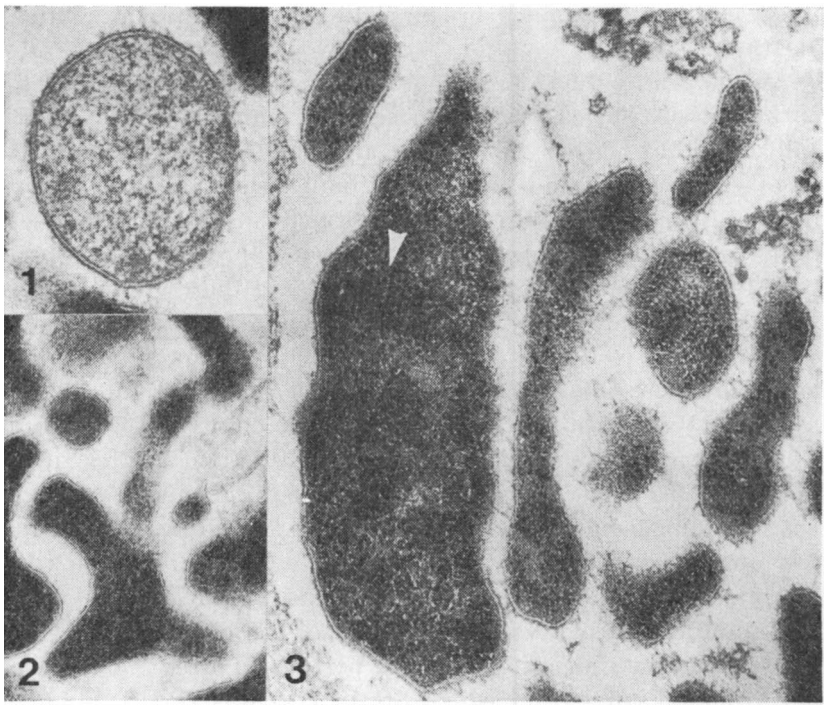

Figure 1. Organism bounded by a clearly visible triple-layered plasma membrane. 70.000 ×.

Figure 2. Highly pleomorphic elements. 40.000 ×.

Figure 3. A group of electron-dense organisms in one of which a pattern of ribosomes arranged in rows can be seen (arrow). 60.000 ×.

It can be concluded that the organisms in question belong to the genus Ureaplasma within the class Mollicutes and probably represent the first finding of organisms of this class in mink. Pathogenic significance of the organisms was not demonstrated.

N. F. Friis and *K. B. Pedersen*
The State Veterinary Serum Laboratory, Copenhagen, and
B. Bloch
The Department of Veterinary Virology and Immunology, Royal Veterinary and Agricultural University, Copenhagen, Denmark.

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Reprints may be requested from: N. F. Friis, the State Veterinary Serum Laboratory, Bülowsvej 27, DK-1870 Copenhagen V, Denmark.