Electronomicroscopically Identification of Rabbit Haemorrhagical Disease Virus

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Abstract. Electronomicroscopy emphasize rabbit haemorrhagical disease virus in lung and liver after experimental infections prove de diagnosis of Hemorrhagic disease of rabbit.

There were used 2 procedures:
♦ Direct negative colored method, obtained from lung and lives from rabbit experimental infected with rabbit haemorrhagical disease virus. (R.H.D.V.)(4).
♦ Positive colored method for detection ultrastructural modification from lungs and liver induced by R.H.D.V.

For both procedures was used a - 125 Sumy EM electronomicroscop. In lung and liver suspension from rabbit experimental infected , electronomicroscopy proved uniform,isometric, unenveloped viral particules, characteristic to calicivirus family.

Key words: electronomicroscopy, lung, liver.

MATERIALS AND METHODS

For detection of ultrastructural modification there we used 2 procedures:

The direct negative colored method ( ME - CND ) obtained from lung and lives triturat, prelevated from rabbit experimental infected with R.H.D.V.(1).

The positive colored method ( ME-CP )(5).

For ME-CND lung and liver fragments were mixed in quart sand (Merck) and PBS (5ml. - pH 7.2-7.4). The obtained suspension obtained was centrifugated 20 minutes at +4C.

From supernatant was prelevated 50 µl and was added electricaly grile of 150 msh covered with double membrane (formwar and carbon)1-2 minutes.

The grilles contrastation was realised with uranil acetat 2% in distilat water, follow by electronomicroscopy examination.

Lung and liver fragments which were prelevated from rabbit experimental infected were fixed for 30 minutes at low temperature, in fosfat alcalin ( TFS) with 2.5% glutaraldehidă.

Later, post fixation ,procedure was done in osmium tetroxid ( OsO4) solution, anhidratated with aethilic alchool (clarificated in propilen oxid  and included in Epon 812).

Slide were obtained with a LKB III ultramicrotom, and were duble contrasted with Reynolds solutions, after their sink in electronic grilles.

For both types, we used EM-125 Sumy electronic microscop - with a 4000 – 60000 times power enlargement(3,1).
RESULTS AND DISCUSSIONS

In organs suspensions (lung, lives) from rabbit experimental infected with V.B.H.I. and which was acute evolution haemorrhagic sindrom, electronomicroscopicaly examen realizated by ME-CND was evidentated more viral particles enanvelopated, izometrical, same size (33-35 nm), with cup forms, wich is characteristic calcivirus family. (picture 1 and 2).

Most size of viral particles was show in lung and lives, where too hemaglutinated test with men hematy O group prezentated hemaglutinant titrus more then 1-256.

Ultrastructural examens from lung and lives, show more electronical condensations perinuclear aglomeration vacuoles from hepatocites and pneumocites (picture 3 and 4).


CONCLUSIONS

R.H.D. virus affects several parenchymatous viscera, but liver and lungs (especially lungs) are most affected.

The highest amount of virus clusters were found in lungs.

This fact can explain the severe pulmonary signs.

REFERENCES