STAGE RAPORT - 2011

During the research stage, following the aimed objectives, the clinical examination of the dogs with renal failure in various degrees was continued (moderate and severe proteinuria). In live patients were performed cytological examinations by echo-guided puncture with thin needle of the echographic detected formations. Thus it allowed the rapid diagnose of pathological processes with renal localisation (congenital cystic lesions, dystrophies, inflammations, renal tumour). In deceased or euthanized dogs, samples were collected, processed, photographed and interpreted after the necropsies.

Histological examination was performed using the paraffin embedding method and routine stains from histopathological examination but also special stains to emphasize the lesions.

The electron microscopically examinations confirmed the suppositions concerning the immune deposit in certain renal structures. It was confirmed the presence of these deposits by immunohistochemistry, using anti-IgG Atc. On the studied cases, the samples were positive, the immune compound involved in generation of the renal failure being confirmed.

The results from cytological, electron microscopically, histopathological and immunohistochemical examinations were corroborated. Was aimed the identification of causes or determining pathological agents for the renal lesions. A data base was settled with all the results obtained during this stage.

The cytological examination of the kidneys in live dogs was performed by echo-guided puncture with thin needle. The dogs were previously anesthetised, the lumbar region was shaved and disinfected for the puncture. The puncture was made by aspiration with a 5 ml syringe with a G22 needle attached. The smears were stained May-Grunwald-Giemsa and examined with optical microscope.

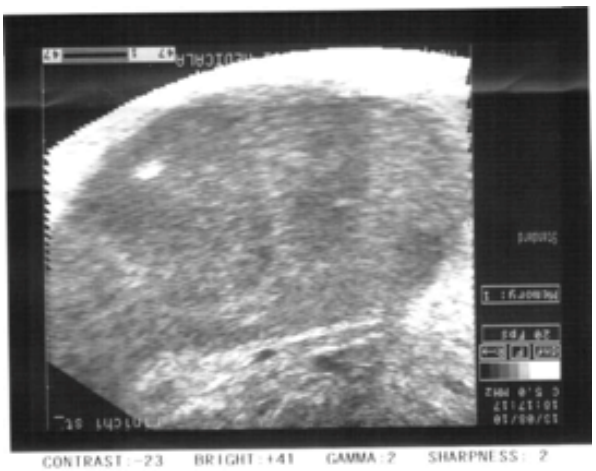

Fig.1 Echo-guided renal cortex biopsy

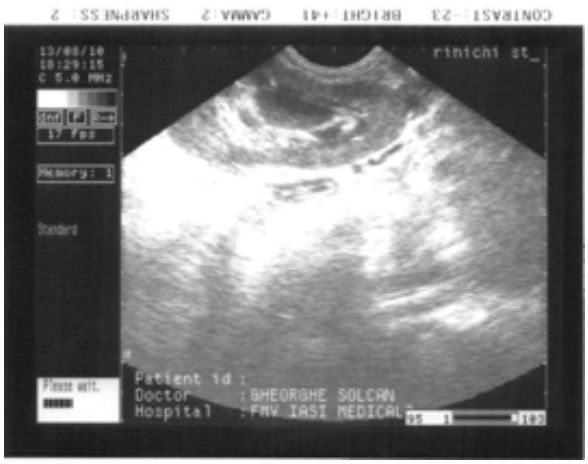

Fig.2 Echo-guided renal cortex-medulla biopsy

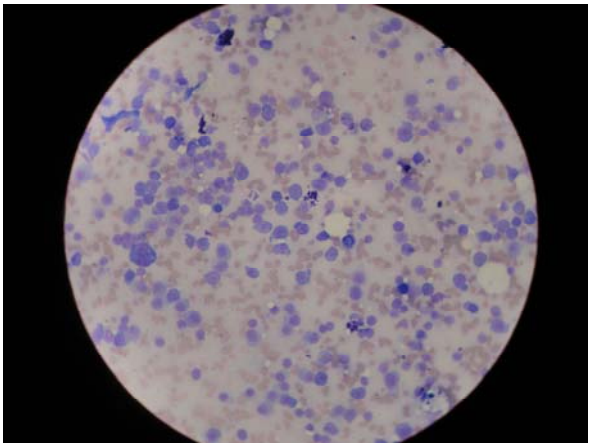

Fig.3 Renal lymphoma. Discrete haemorrhagic fond, round polymorphic cell population, with slightly basophilic vacuolated cytoplasm with round nucleus. Cellular pleiomorphism (anizocytosis, anizocariosis). Bi- or multinuclear cells. Atypical frequent mitosis. Rare granulocytes, neutrofiles, eosinophils and mastocytes. Col. MGG, x1000;

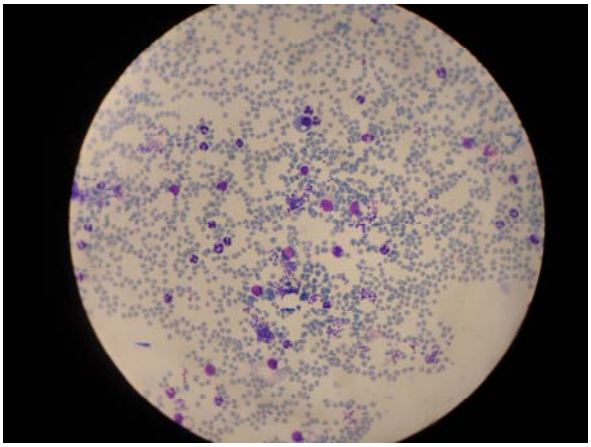

Fig.4 Dog's kidney. Renal steatosis. Rare normal nephrocytes and discretely vacuolated. Col. MGG, x1000;

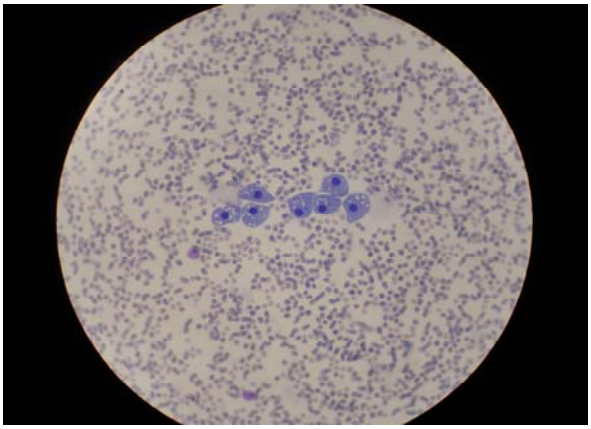

Fig.5 Dog's kidney. Renal steatosis. Epithelial cells isolated or grouped with lipid vacuoles inside cytoplasm. Col. MGG, x1000;

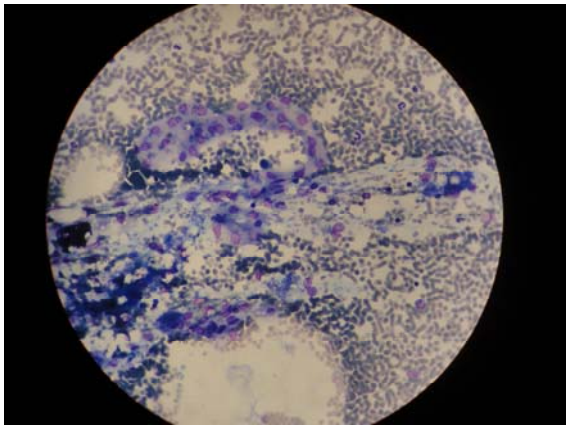

Fig.6 Renal mastocytome. Round cells with round or oval well emphasized nucleus. Cytoplasmatic granulations, violet colour, round, small in dimensions. Col. MGG, x1000;

To emphasize the immunoglobulin involved in evolution of glomerulonephritis, were selected 30 cases (dogs) with acute and chronically disorders, from which mostly were not having clinical signs of renal failure. The tissue specimens were fixed in 10% formaldehyde and sectioned at 5μm.

Immunohistochemistry technique

After placing the specimen on the glass slide, the samples were cleaned of paraffin (in 3 baths with xylene for 20 min) and hydrated (in 3 baths with absolute alcohol 90^0, 80^0 for 10 min).

The section were washed in PBS (phosphate buffer saline solution, pH – 7,2), then the endogenous peroxidises were blocked by incubation with a mixture of tap water and oxidised water (H_2O_2) 3%, v/v, for 5 min.

The sections were incubated at 22^0C for 10 min with prediluted blocking horse serum (NHS). After removal of the exces of blocking serum, without wash, the sections were incubated for 30 minutes with first mouse antibody IgG (Mouse Monoclonal Antibodies Immunoglobulin G Leica) diluted 1/300 plus 5% blocking ser. The sections were washed energically for 5 min. in PBS.

On sections was added the second antibody anti-specie (Novocastra Prediluted Biotynilated Universal Secondary), for 10 min., at 22^0C.

Again, the sections were washed for 5 minutes in PBS. The sections were incubated with streptavidine/peroxydase enzymatic complex for 10 minutes. Again the sections were washed in PBS for 5 min. The last stage is incubation in chromogene substrate (DAB – 3.3´di-amino benzidine Novocastra Leica) for 5 min. The sections were washed again with tap water.

The sections were successively dehydrated, by absolute alcohol 80^0, 90^0 baths for 10 minutes and finally clarified with xylene and fixed. The stain was made with Mayer haematoxylin (Merck) for 5 minutes.

The IgG on glomerular structures was of dark brown colour.

The electronmicroscopically examination

The electronmicroscopically investigations were performed with the help of electronic microscope with TESLA BS 500 transmission. The samples were prepared as described:

1. Collecting: tissue specimens were collected with a volume of 1 mm^3 from kidneys (cortex and medulla) on a drop of 2% glutaraldehyde in phosphate buffer.

2. Prefixation was performed in 2% glutaraldehyde in phosphate buffer, 2 hours at 4^0C.

3. Wash of the samples was performed with phosphate buffer , 2 hours at 4^0C.

4. Fixation of the samples with 1% osmium tetra-oxyde in phosphate buffer.

5. Wash of the sample was made with phosphate buffer, 2 hours at 4^0C (the phosphate was changed 2-3 times).

6. Dehydration was made with 30% alcohol, 20 minutes at 4^0C;

→ mass staining was made with uranyl acetate in 30% alcohol, 45 minutes at 4^0C

- 30% alcohol, 10 minutes at 4^0C
- 50% alcohol, 20 minutes at 4^0C;
- 70% alcohol, 20 minutes at room temperature;

→ mass attaining was made with fosfotungstic acid in 70% alcohol, 45 minutes

- 70% alcohol, 10 minutes at room temperature;
- 90% alcohol, 20 minutes at room temperature;

7. Infiltration was performed by passing the samples through 3 successive baths with absolute alcohol, 20 minutes / bath and 3 successive baths with acetone, 20 minutes/ bath.

8. Impregnation – was made with EPON 25% + acetone 50%

9. For inclusion were used special capsules for inclusion, with EPON.

10. Polymerization – the capsules with the sample included in EPON were kept in incubator at 60^0C for 3 days.

11. Trimming

12. Sections: on ultramicrotone, the section has 60-90 nm thickness

13. Laying on grills covered with formvar pellicle.

14. Staining:

- uranil acetate sol.: 10 minutes,
- Reynolds sol.: 3-5 minutes.

After their preparation, the grills with samples are introduced in electron microscope for examination.

The kidney is often the victim of aggression with extra renal origins. Its structure and its function make it vulnerable and part of changes with pathological character developed inside the living organism.

The increased sanguine pressure from the glomerular capillaries, the ultra filter role and the glycoproteins with increased negative charge from the structure of the filtering glomerular barrier pleads in favour of susceptibility to potentially toxic action of the circulating endogenous or exogenous substances.

Taking into account the induction mechanism, the immune glomerulonephritis may be separated in two categorie:

1.The glomerulonephritis produced by the immune complexes accumulated in glomerule;

2. The glomerulonephritis produced by anti-basal membrane antibodies.

From histological point of view, in examined cases, were emphasized all histological types of glomerulonephritis (membranous gl., membranous proliferative gl., mesangioproliferative gl.).

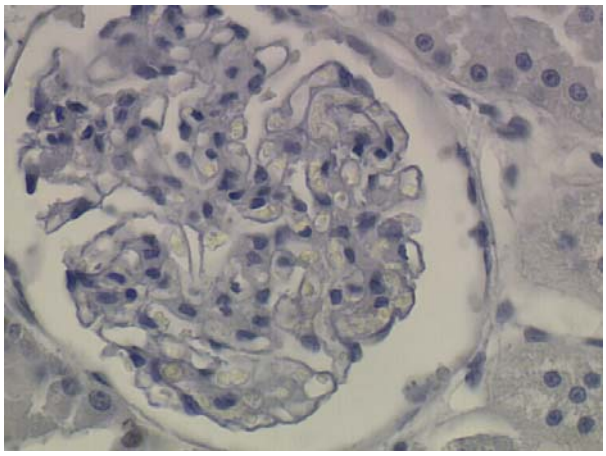

Fig.7 Dog's kidney. Membranous glomerulonephritis. Col. Mayer Haematoxylin, x900;

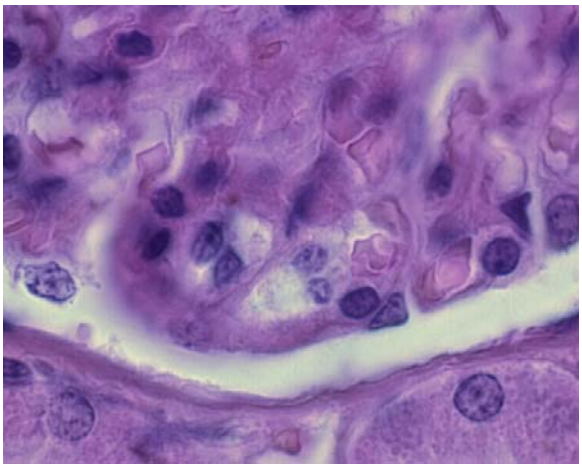

Fig.8 Dog's kidney. Membranous glomerulonephritis. Thickened glomerular capillaries. Col. PAS, x1600;

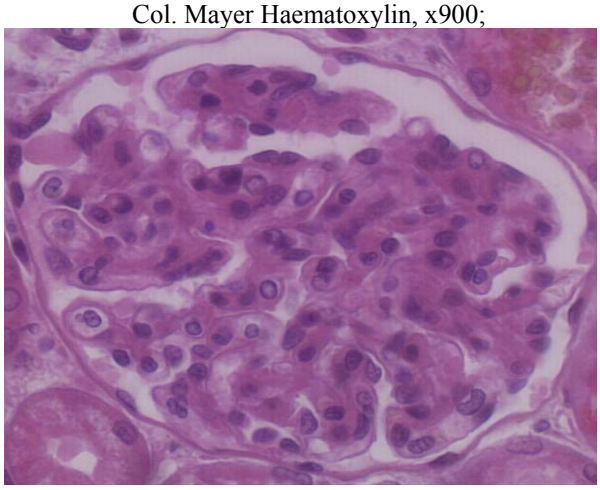

Fig.9 Dog's kidney. Mesangioproliferative glomerulonephritis. Col. HE, x900;

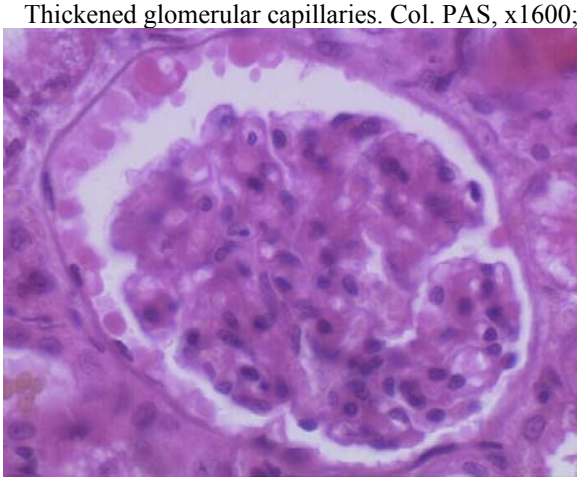

Fig.10 Dog's kidney. Membrane-proliferative glomerulonephritis. Col. PAS, x900;

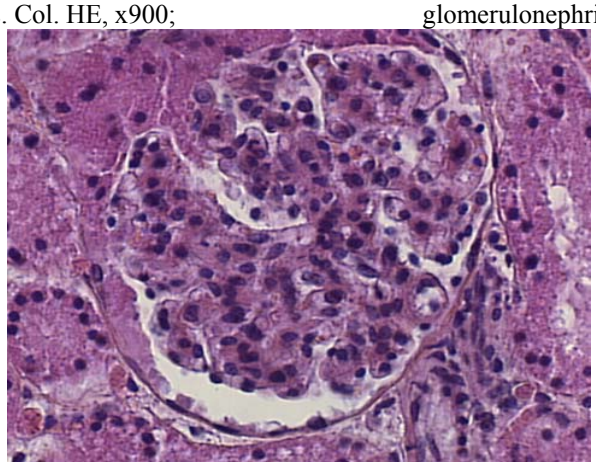

Fig.11 Dog's kidney. Membrane-proliferative glomerulonephritis. Col. HE, x900;

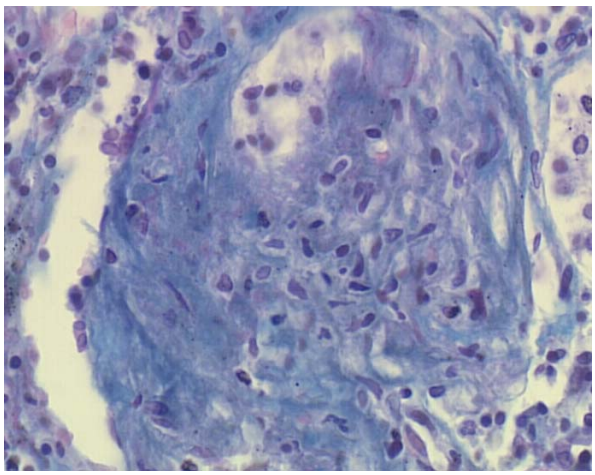

Fig.12 Dog's kidney. Sclerosis glomerulonephritis. Col. tricromic Masson, x900;

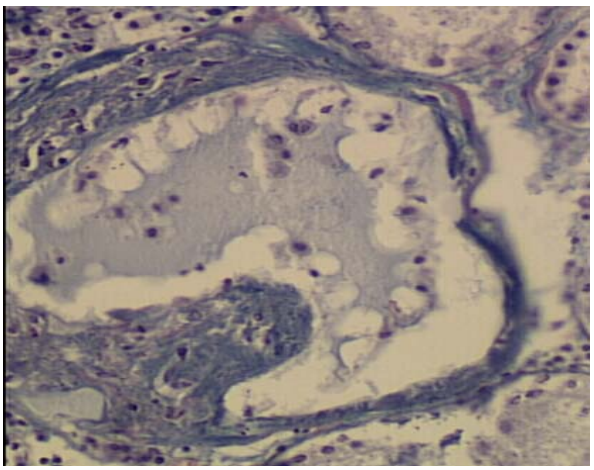

Fig.13 Dog's kidney. Sclerosis glomerulonephritis. Col. tricromic Masson, x900;

At histological examination was underlined the G immunoglobuline mark inside the glomerular structures with granular and linear aspect. These were localised mostly at the level of the glomerular mesangiom.

The cases where was emphasized the G Ig in kidneys, are dogs that developed all three types of immune glomerulonephritis (membranous gl., membranousproliferative gl., mesangioproliferative gl.), identified by normal histological examinations (paraffin embedding and HEA stain).

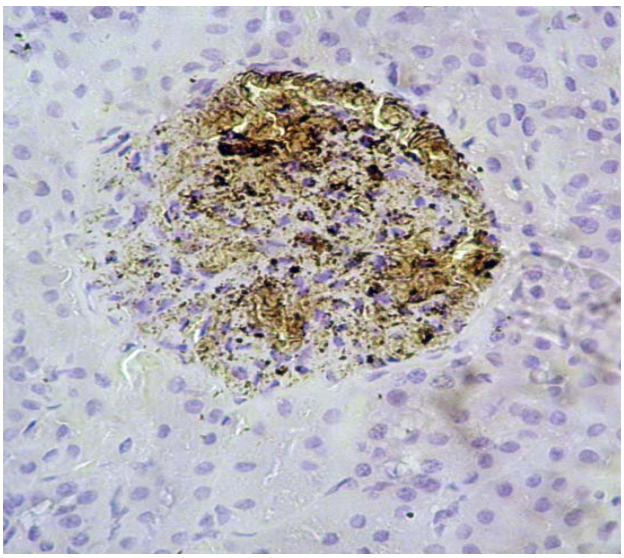

Fig.14 Dog's kidney. IgG mark. Col. Mayer Haematoxylin, x400;

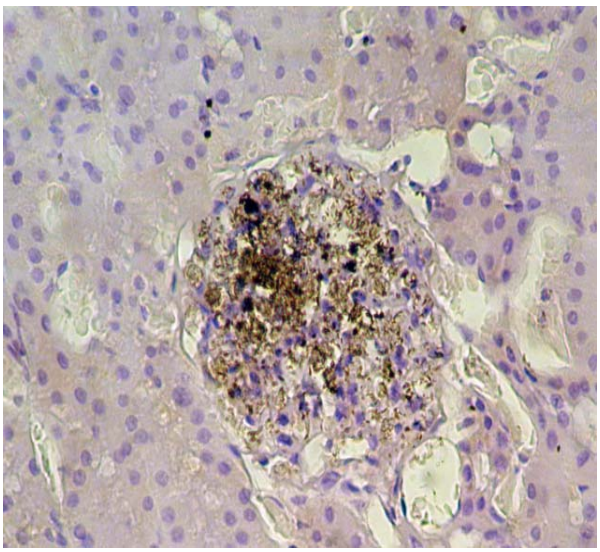

Fig.15 Dog's kidney. IgG mark. Col. Mayer Haematoxylin, x400;

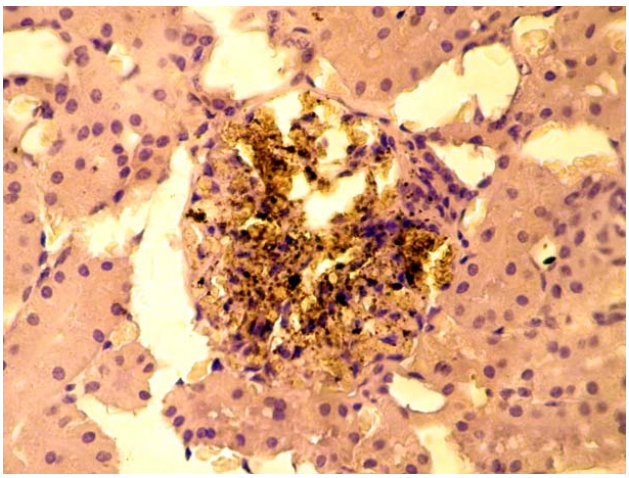

Fig.16 Dog's kidney. IgG mark.
Col. Mayer Haematoxylin, x400;

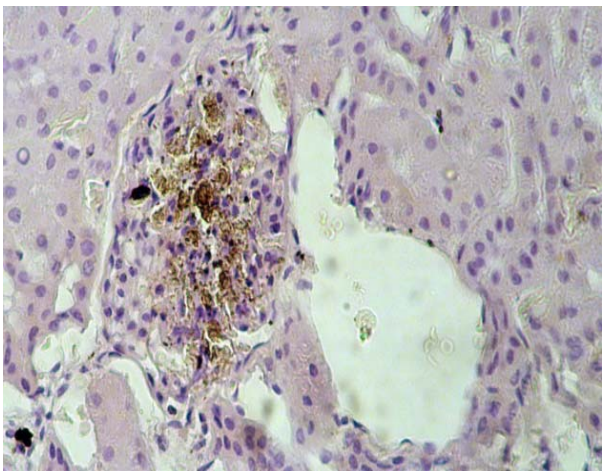

Fig.17 Dog's kidney. IgG mark.
Col. Mayer Haematoxylin, x400;

Electron microscopic examination emphasized the thickening of the basal membrane of the glomerular capillaries (glomerulosclerosis) and the presence of electron dense deposits with spiculi aspect on internal side (sub endothelial) and external side (sub epithelial).

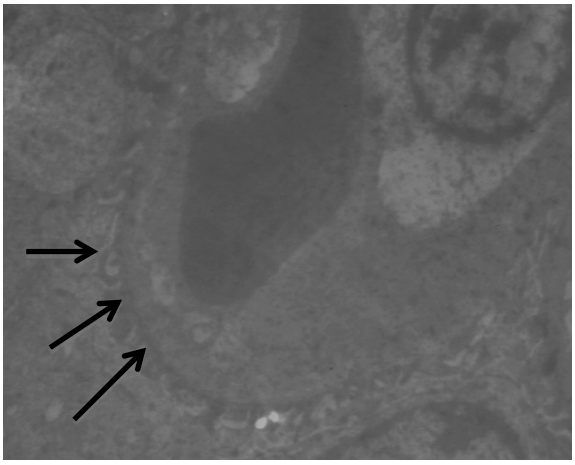

Fig.18 Dog's kidney. Glomerular capillary. Sub epithelial immune deposits with spiculi aspect. TEM, X2400;

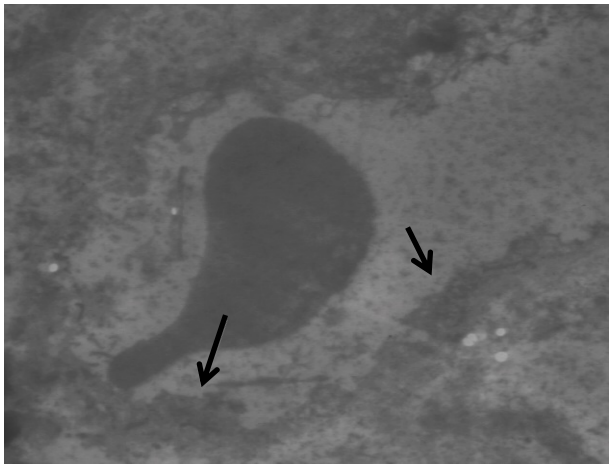

Fig.19 Dog's kidney. Glomerular capillary. Sub epithelial immune deposits with spiculi aspect. TEM, X2400;

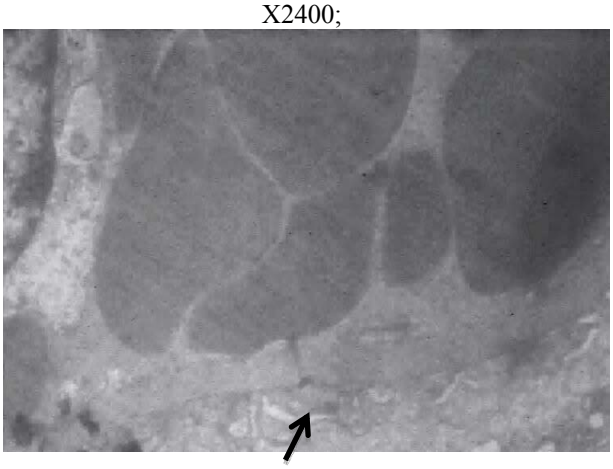

Fig.20 Dog's kidney. Glomerular capillary. Sub epithelial immune deposits with spiculi aspect. TEM, X2400;

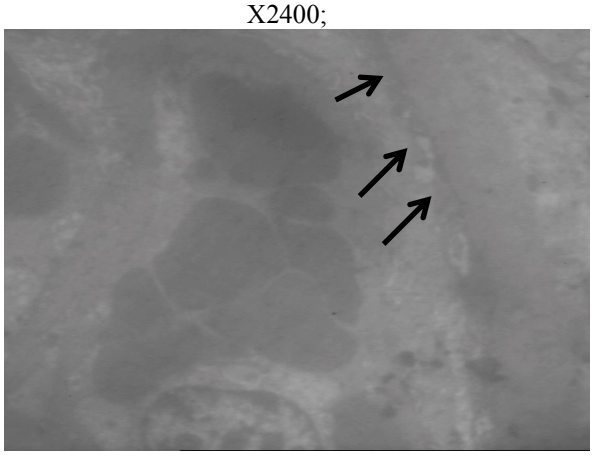

Fig.21 Dog's kidney. Glomerular capillary. Sub epithelial immune deposits with spiculi aspect. TEM, X1250;

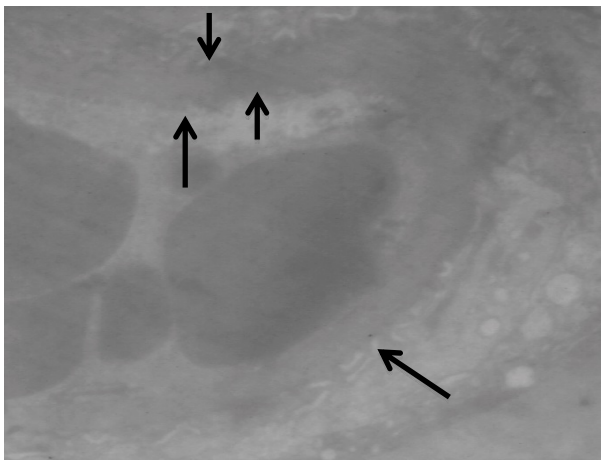

Fig. Dog's kidney. Glomerular capillary. Sub epithelial immune deposits with spiculi aspect.
TEM, X2400;

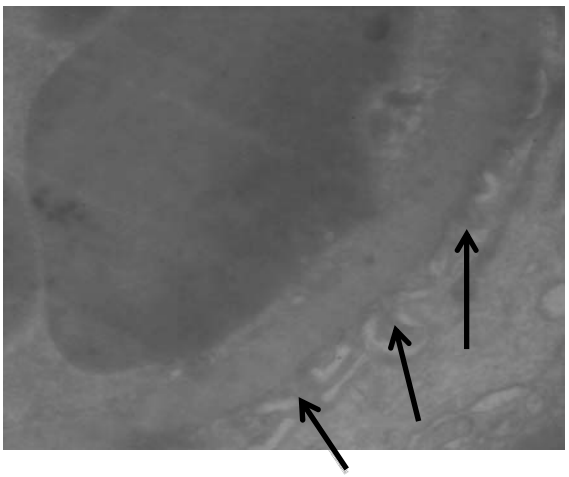

Fig. Dog's kidney. Glomerular capillary. Sub epithelial immune deposits with spiculi aspect.
TEM, X4400;

In induction of the immune glomerulonephritis, Atg-Atc immune circulatory complexes represent the main factor.

These complexes may contain bacteria, viruses, parasite antigens or neoplasm antigens.

The pathogenesis and the way the immune complexes accumulate on glomerular structures depend on quantitative and qualitative aspects:

- Atc-Atg quantity of complexes;
- the dimension of complexes;
- molecular configuration;
- the antibody affinity to antigen;
- electrical charge;
- solubility.

The big complexes, insoluble and excessively formed are rapidly removed from blood at kidney level and phagocytised by monocytes-macrophage system (MMS) or partially taken by the glomerular mesangium.

On the other hand, the immune complexes by intermediary dimensions formed in presence of excessively antigens remain in liquid and may accumulate on basal membrane of the glomerular membrane, of the rounded tuft of blood capillaries or at the level of mesangium.

By the mean of the electron microscopy the accumulated immune complexes are emphasized under the shape of unregulated deposits, electron-dense disposed in subendothelial position (*subendothelial GN*) or subepithelial (*subepithelial GN*), in thickness of the basal membrane (*intramembranous GN*) or in mesangium (*mesangial GN*).

The deposit in subendothelial position is usual for the circulatory immune complexes with increased anionic charge that is not allowing the passage through glomerular basal

membrane and with high affinity of the antibody for antigen that makes them difficult to dissociate.

On the contrary, the subepithelial position is usual for the complexes with cationic charge and with low affinity of the antibody to antigen, which allows the dissociation of the immune complex and independent migration of the antigen and antibody through glomerular basal membrane, reforming the complex on its subepithelial side.

The intra-membrane deposit of the immune complexes is less usual, in some cases representing an intermediary phase of migration through thickness of the glomerular basal membrane.

The evolution of the observed glomerulonephritis was induced by deposit in glomerular structures of the G immunoglobulin, immunohistochemistry permitting to the researcher to emphasize the regulated, homogenous and granular deposit placed subendothelial along the glomerular basal membrane.

These proliferative phenomena have as consequence the segmental glomerular sclerosis and even generally glomerular sclerosis, failure of the glomerular capillaries and adhesions between the rounded tuft of blood capillaries and Bowman's capsule.

The quantitative and qualitative evaluation of these lesions is indispensable in the histological prognostic.