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Artikel: Histopathological, immunohistochemical and electron microscopic methods for the diagnosis of fox distemper infection

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the contents of alveoli have been reported as phagozyted transudates due to increased capillary permeability.

The budgerigar may be an adequate spontaneous animal model for research in human endogenous lipid pneumonia.

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HISTOPATHOLOGICAL, IMMUNOHISTOCHEMICAL AND ELECTRON MICROSCOPIC METHODS FOR THE DIAGNOSIS OF FOX DISTEMPER INFECTION

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Distemper virus (DV) is a Morbilivirus pathogenic for dogs, minks, foxes and others Mustelidae that produces different clinical diseases according to the strain of virus, virus dose, susceptibility and immune response of the host (2, 3, 7). DV is highly transmissible and 20–80% of the infected animals die (1, 4).

DV – air-borne first multiplies in the lymphoid tissue and then spreads by the leukocytes 8–9 days post infectionem to the epithelial and nervous cells (1).

Several morphological methods are used for the identification of DV infections like histopathological demonstration of inclusion bodies or immunohistochemical identification of viral antigen (5, 8, 9).

The purpose of this study was to report the distribution of inclusion bodies and viral antigen in non vaccinated naturally infected foxes by means of histopathological, immunohistochemical and ultrastructural techniques.

Material and methods

Samples of lungs, trachea, spleen, lymph nodes, kidney, urinary bladder and nervous system were obtained from 8 foxes naturally infected with DV, and fixed in 10% phosphate buffered formalin and 2.5% buffered glutaraldehyde.

Tissues were processed according to routine histological methods, embedded in paraplast and stained by H-E and Shorr-S3 stainings. An indirect immunoperoxidase (IPI) method was used to detect DV antigen, using as first antiserum a monoclonal antibody against the nucleocapsid protein (10).

Samples of renal pelvis epithelium – fixed in glutaraldehyde – were processed according to routine methods for transmission electron microscopy.

Table 1: Comparative results IB/IPI

Results	1 2 3 4 5 6 7 8
Respir. IB	+ + + – – + – –
system IPI	+ + + + + + + +
Lymph IB	+ 0 0 0 0 0 0 –
organs IPI	+ 0 0 0 0 0 0 +
Urinary IB	– + + – – – – –
system IPI	– + + – + – + +

IB inclusion bodies
IPI indirect immuno-peroxidase
+ positive; – negative; 0 no samples

Histopathological lesions. The most frequent lesion observed was interstitial pneumonía with dilatation of the interstitium due to infiltration of mononuclear cells. Trachea and bronchi presented intracytoplasmic and intranuclear inclusion bodies. Lymphatic organs were characterized by necrosis and by the appearance of eosinophilic intracytoplamic and intranuclear inclusions. Moderate to severe congestion was shown in the urinary bladder. Intracytoplasmic and intranuclear inclusions were detected in the epithelium of the urinary bladder and renal pelvis. No other significant lesions were present in the kidney.

Immunocytochemical results. The positive cells to the IPI showed a cytoplasm with dark-brown granules, the nuclei were not involved. Peroxidase-positive material was found in alveolar cells, interstitial cells, macrophages, trachea epithelial cells (Fig. 1), trachea gland cells, bronchial and bronchiolar epithelial cells, lymphoid cells, macrophages of the lymphoid system and epithelial cells of the urinary bladder (Fig. 2) and renal pelvis. In one case positive material was found in glomerular mesangial cells.

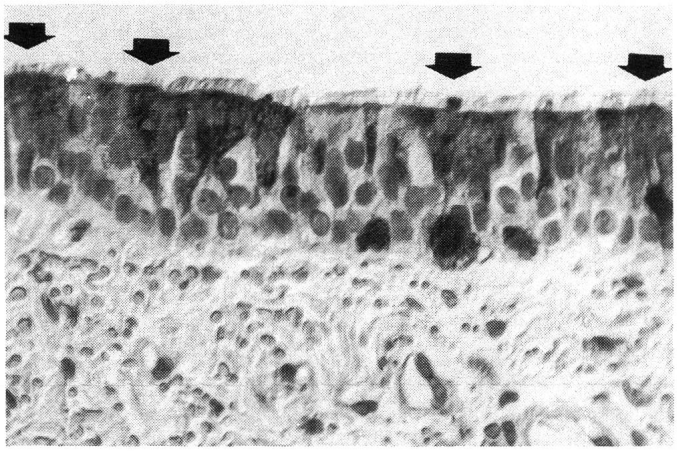

Fig. 1: Trachea. Cytoplasm peroxidase-positive material in the epitelial cells (arrows). IPI. X400

Table 1 summarizes the histopathological and immunohistochemical findings. *Ultrastructural results.* Particles like DV were found in the cytoplasm of the epithelial cells of the renal pelvis.

Discussion

In this study distemper infection was diagnosed by evidence of inclusion bodies, immunoperoxidase techniques and electron-microscopic procedures.

Inclusion bodies (IB) were traditionally associated with distemper infection, but it was verified that occasionally IB were not identified

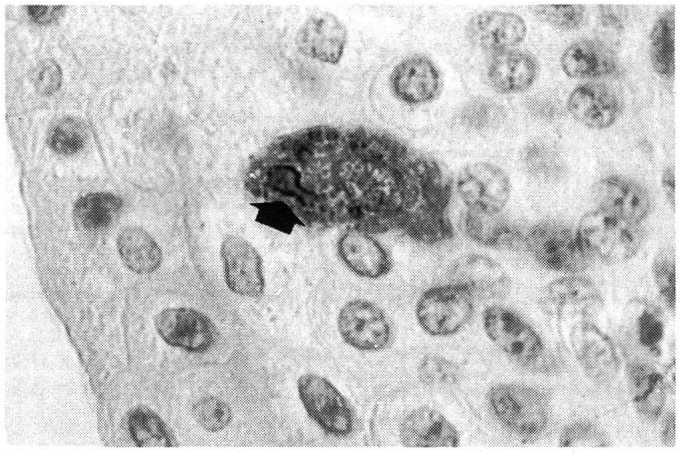

Fig. 2: Urinary bladder. Peroxidase positive material and inclusion bodies (arrow) in the transitional epithelium. IPI. X600

by immunohistochemical methods (5, 8, 9). Such inclusions may represent residual cellular matrix or herpes simplex inclusions (6).
Immunocytochemical techniques have been used for the demonstration of DV antigen in tissues of dogs (5, 8, 9) and minks (3). The detection of positive reacting tissues, using a monoclonal antibody against canine DV in foxes, suggested that the same agent is the cause of the infection or that DV in foxes shows cross-reactivity. The detection of positive reacting tissues that were negative for inclusion bodies was also reported in dogs (5) and can be explained by the late appearance of inclusion bodies or by the decrease of these inclusions 15–20 days post infectionem (6).
Respiratory, lymphoid and urinary organs were positive to the detection of DV antigen in the present study. No lesions or positive reaction were found in the nervous system.
By electron-microscopic studies we have demonstrated intracytoplasmic DV-like particles in the epithelium of renal pelvis. This technique provides the evidence of distemper infection but it is time-consuming and needs a special procedure not always available in a diagnostic histopathological laboratory.
In conclusion, distemper infection in foxes could be proved efficiently by the combination of histopathology and immunohistochemistry. The immuno peroxidase procedure is more efficent and specific than the evidence of inclusion bodies.

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A SIMPLE PROCEDURE FOR THE GROSS AND HISTOLOGICAL EXAMINATION OF THE BOVINE HOOF.

P. Ossent

The method is based simply on the removal of the whole horn shoe. In spite of its simplicity and efficiency, and although every pig undergoes essentially the same process at slaughter, the technique has hardly been used for the examination of bovine claws. Nilsson (1) and Maclean (2), in the early 1960's, exungulated bovine claws but apparently did not make use of hot water, which greatly reduces artifact. The usual procedure for the inspection of the inner regions of the claw at routine necropsy as well as in the literature is simply to saw a sagittal section of the hoof. In the bovine, this delivers nearly no information.
First, the foot is cleaned and the solar surface of the claw is pared to expose any heamorrhages or defects. Next, the foot is immersed to the level of the coronary band in hot water for several minutes until the inner temperature reaches approx. 60°C. The horn shoe is then removed with the help of a vice.
The exposure of the complete surface of the corium and the inner surface of the horn shoe presents a correspondingly complete picture of the situation. It allows specific tissue sampling for histology from e.g. focal lesions which is otherwise impossible. The line of separation between shoe and its substrate is usually in the str. spinosum, so the transitional zone may be studied microscopically. The deeper layers of the dermis and the surface of the claw bone are easily accessible, as is the bone itself which may be macerated and cleaned to expose any defects at that level too.
Indeed, the method's application is not restricted to the necropsy room. The simplicity of the method allows it to be carried out on the farm yard. This may be indicated in slaughtered animals with e.g. acute and subacute laminitis, where a marked discrepancy between the lack of visible changes in the live animal and the very pronounced interior lesions is common. The practitioner may demonstrate the severity of the lesions to the owner and thus remove any possible vestiges of doubt that the decision to slaughter the cow was justified.

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