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alterations in experimentally infected animals are not well known yet either. Additionally, experimentally infected cats usually do not have the same burden of opportunistic infections or other environmental stresses present in free roaming spontaneously infected animals.

Thus, at the moment only associations between FIV-infection and lesions frequently occurring in these animals can be described. Since FIV-positive cats are usually older than the average cat population at necropsy it is also difficult to differentiate between age-associated and FIV-associated findings. FIV-positive cats are often destroyed when the diagnosis «FIV infection» is made. Statistical evaluation of FIV-associated lesions is blurred by the fact that false positive as well as false negative results occur in ELISA. Furthermore, the cats are destroyed or die when being in different phases of the infection (acute phase, asymptomatic carrier, ARC, AIDS) which may result in completely different alterations (e.g. follicular hyperplasia and dysplasia in lymph nodes versus hypocellular follicles and nodes). We had the opportunity to investigate carcasses and/or tissues of 25 cats for whom FIV-infection was diagnosed by ELISA and which were sent to the Department of Veterinary Pathology, School of Veterinary Medicine, University of Giessen. The following findings appear to be circumstantial evidence for an FIV-infection.

Cats older than 6 years of age and male or male neutered animals predominate at post mortem investigation as also reported from clinical studies. The case history most often includes stomatitis, diarrhea, pyrexia, emaciation, rhinitis or cellulitis.

There are no specific alterations at necropsy in FIV-infected cats. Anemia, emaciation, stomatitis and rhinitis, lymphoid hyperplasia and nephritis are found most often.

Histologically the most consistent and striking fact is hypercellularity of bone marrow which even in old animals is easily diagnosed in the marrow of the femur. The majority of hypercellular bone marrows also display a predominance of immature nuclear forms consistent with myelodysplasia. In the brain there is often a mild to moderate localized encephalitis and/or meningitis. The encephalitis preferentially occurs in the cerebral cortex. The kidneys exhibit in the vast majority of FIV-positive cats interstitial lesions ranging from chronic focal interstitial nephritis to nephrosclerosis. Additionally, there is a usually focal and segmental but sometimes also panglomerular and diffuse glomerulonephritis found in most cases. The lymphatic tissues of spleen and lymph nodes often show proteinaceous and/or fibrillar material with or without clefts covered by endothelial-like cells in follicles. Often follicles are hypocellular or inconspicuous but sometimes there is hyperplasia of follicles and follicles are found in the medulla of lymph nodes. Lymph nodes often show proliferation of activated macrophages in the sinus. Fat storage in Ito cells and hepatocytes, cholangitis and pericholangitis as well as bile duct proliferation is often present in the liver. Bilirubin storage in hepatocytes also occurs frequently. In the intestine moderate to severe cellular infiltration of the mucosa, mild depletion and dilatation of crypts, crypt epithelium degeneration and crypt abscesses are found in some animals.

At the moment there is no way to diagnose FIV infection by post mortem investigation unequivocally. None of the alterations mentioned above is specific for a FIV-associated disease in cats. Combinations of these findings, however, allow for the interpretation that FIV infection may have been the primary cause of disease in the animal.

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### **OVINE LENTIVIRUS INFECTION: ARTHRITIS IN SHEEP RELATED TO VIRUS ISOLATION BY SYNOVIAL MEMBRANE EXPLANTS**

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Maedi/Visna is a disease of mature sheep caused by a lentivirus closely related to caprine arthritis-encephalitis (CAE) virus, even though differences between the genome sequences of the two viruses are reported.

Arthritis is a typical lesion in CAE, but it is not a common finding in lentivirus infection of sheep. Oliver R. E. et al. (1981) and Cutlip R. et al. (1985) described arthritis in sheep with ovine progressive pneumonia, the equivalent of Maedi described in USA.

In the present report we describe carpal joint lesions in sheep positive to Maedi/Visna by the agar-gel immunodiffusion (AGID) test for Maedi/Visna. Our observations were made during an attempt to eradicate Maedi infection in two flocks with low prevalence of reactors.

#### **Material and methods**

Three ewes, between 3 to 4 years of age without symptoms of disease, were subjected to necropsy. The first was a Garfagnina ewe (A) from

the Experimental Farm of Orecchiella park (Lucca, Italy), the second and third (B) were from a private farm in Siena (Italy).

Samples of lung, mammary gland, choroid plexus and synovial membrane were collected and processed for virological and histopathological examinations to verify the specificity of serological reaction. Explants of collected tissues were cultured in 25 cm<sup>2</sup> flasks containing MEM supplemented by 15% bovine fetal serum. When the cellular layer was confluent, cells were passaged at weekly intervals and sowed in chamber slides (Miles-Illinois). Slides were stained with Giemsa after one week from the passage.

Cell cultures in 75 cm<sup>2</sup> flasks were scraped and centrifugated at 1000 g for 18 minutes. Pellets were fixed in 3% glutaraldehyde, post-fixed in O<sub>3</sub>O<sub>4</sub> and embedded in Epon-Araldite. Semi-thin sections were stained with methylene blue and ultra-thin sections with uranyl acetate and lead citrate for TEM observation.

Tissues for light microscopical examination were fixed in buffered formalin (pH 7.3), embedded in paraffin and sectioned at 5 µm. Sections were stained with hematoxylin and eosin, Van Gieson and phosphotungstic acid hematoxylin (PTAH).

## Results

All explants resulted in a confluent monolayer within 2–4 weeks. Syncytia were not observed in explants of lung, mammary gland and choroid plexus of ewe (A). Explants of synovial membrane showed syncytia with the typical circular arrangement of nuclei. Syncytia were detected on the second passage of the cells and increased in number and size with further passages.

Syncytia were consistently present in the explants of mammary gland of the (B) group of ewes but not in explants of lung, synovium and choroid plexus.

The liquid of infected cultures was concentrated (120x) by dialysis against polyethylene glycol, and used as antigen in AGID test against sheep anti-Maedi/Visna positive serum, obtaining a specific precipitation line.

This result, in addition to the biological properties *in vitro*, permitted to identify the viral isolate as Maedi/Visna virus although comparisons of the nucleotide sequence of the isolate with other ovine lentivirus strains were not performed.

Bacteriological examinations excluded the presence of chlamydia and mycoplasma in the collected tissues.

At necropsy moderate articular lesions with oedema, periarticular carpal tissue thickening, hyperemia and synovial hypertrophy were seen. Induration of mammary gland was ascertained in the animals of (B) group. The lungs did not show the typical changes of lentivirus pneumonitis.

At microscopical examination pulmonary lesions were not seen in any case. Interstitial mastitis with lymphocytes, plasma cells and

macrophages arranged in pseudofollicular patterns around galactophorous ducts was evidenced in the ewes of the (B) group.

Articular histopathological lesions were oedema, hyperplasia and hypertrophy of synovium villi and severe infiltration of lymphocytes and plasma cells. Phlogistic cells were also present near the tendon sheaths, sometimes arranged in periarteriolar cuffs. Arteriolar parietal cells were somewhere changed by regressive phenomena. Hyalin degenerative lesions were sometimes ascertained in the connective tissue; necrosis was rare. Recent and older hemorrhagic focal lesions with presence of macrophages filled with hemosiderin were also seen. Frequently were observed few foci of mineralization in the synovium connective tissue. In the semi-thin sections were ascertained syncytia, cytoplasmic micro-vacuoles and chromatin scattering. Electron microscopy demonstrated mature and budding viral particles morphologically identical to ovi-caprine lentiviruses.

## Discussion

Chronic arthritis and synovitis in Maedi/Visna is a rare finding in sheep. We point out in our cases the particular interest of Maedi arthritis with or without mastitis but always without pulmonary lesions as the predominant pattern.

Moreover the virological studies strengthen this observation since the virus was isolated by synovium and mammary gland, but not by lung and choroid plexus explants, with a positive correlation between virus isolation and tissue injury.

## References

1. *Cutlip R. D.* et al. (1985): *Am. J. Vet. Res.* 46, 65–68.
2. *Oliver R. E.* et al. (1981): *Am. J. Vet. Res.* 42, 1560–1564.

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## APPLICATION OF IMMUNOHISTOCHEMISTRY IN CANINE NEURO-ONCOLOGY

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In the past twenty years, the identification and biochemical characterization of proteins related to the central nervous system (CNS), coupled with the development of hybridoma technology, have resulted in the development of high specificity antibodies and more refined immunohistochemical methodologies for use with formalin-fixed, paraffin-embedded sections. Nervous system markers include glial fibrillary acidic protein (GFAP), myelin basic protein (MBP), myelin-associated glycoprotein (MAG), S-100 protein, the three subunits of neurofilaments (NF's), and neuron-specific enolase (NSE). In addition, cell markers such as cytokeratins (CK's), vimentin, desmin, oncodevelopmental antigens, photoreceptor proteins (retinal S-antigen and rhod-opsin), Leu-7, B and T lymphocyte surface antigens, Factor VIII-related antigen (FVIII-RA), immunoglobulins, and an array of enzymes, pituitary and hypothalamic hormones, and neurotransmitters have been shown to be useful in diagnostic human neuro-oncology.<sup>1,2</sup>

Among domestic animals, the dog has the highest frequency of primary CNS neoplasms and the broadest spectrum of tumours, the majority of which share morphologic similarities with their human

counterparts.<sup>3,4</sup> Although a significant number of these neoplasm are diagnosed with the hematoxylin and eosin stain, approximately 5–10% may be difficult to classify. Reports on immunohistochemistry in veterinary diagnostic neuro-oncology have been relatively few and limited to canine CNS tumours.<sup>5,9</sup> Vandeveldel et al.<sup>6</sup> used GFAP, MBP, and MAG antibodies on 74 canine neuroectodermal tumours. Ten of 16 astrocytomas, one of nine ependymomas, two of three glioblastomas, and 9 of 19 poorly differentiated gliomas stained diffusely or focally with GFAP. Oligodendrogliomas reacted with MAG (three out of 11), but not with MBP. In addition to GFAP, canine ependymomas may also stain with S-100, and cytokeratin, the latter marker usually present in anaplastic ependymomas.<sup>7</sup> However, lack of staining of ependymomas with GFAP has been reported.<sup>8</sup> Alpha-fetoprotein have proved useful in the identification of suprasellar germ cell tumours in the dog.<sup>9</sup> Canine choroid plexus papillomas may express epithelial differentiation (three out of 11),<sup>10</sup> but do not stain with GFAP.<sup>6,10</sup> A thoracolumbar spinal cord neoplasm in young dogs with a tendency for epithelial differentiation has been consistently positive for CK.<sup>8,11,12</sup> Staining with vimentin occurs with