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indirect influence the other infections had on the occurrence of plexus lesions.

In conclusion, the irregular occurence of slight to moderate axon lesions in the intestinal plexus submucosus of pigs colonized by E.

coli O139:K12(B):H1 was not correlated with acute ED or asymptomatic bacterial colonisation, respectively. However, the fact that nearly all pigs had concurrent infections possibly affecting the plexus submucosus, has to be considered.

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EXPRESSION OF CYTOKERATINES IN EPITHELIAL TUMOURS OF THE DOG INVESTIGATED WITH MONOCLONAL ANTIBODIES

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Cytokeratines (CKs) represent a family of 19 polypeptides (Moll et al., 1982). In various epithelia and varying conditios, subsets of 2–10 polypeptides are expressed (differential expression of CKs). It also emerged from numerous investigations that doublets of CKs are preferentially coexpressed (concept of CK pairs), reflecting the functional significance of each CK polypeptide. This functional significance is reproduced with great fidelity in pathological conditions. Consequently, CK typing is a powerfull tool in epithelial cell typing (Sun et al., 1984).

Here we communicate about the CK content of skin epithelial tumours of dogs, i. e. squamous cell carcinoma (SCC), cornifying epithelioma (CE) and basal cell tumour (BCT).

Material and methods

Five SCC, 3 CE, and 6 BCT cases were included in this study. Excised tumours were divided into pieces: one for routine histopathological examination and two pieces for the immunohistochemical study (unfixed tissues, 6μm cryoslices, indirect peroxidase technique: Broekaert et al., 1988). In addition to the monoclonal antibodies listed by Broekaert et al. (1988), we regulary included the following ones in our panel: 21D7 (anti CK5), M20 (anti CK8), LPH2 (anti CK10), PAB601 (anti CK14), LL026 (anti CK16) and the broad spectrum probe LP34.

Results and discussion

CK reactivity profiles are summarized in Table 1. Furthermore, all tumours were positive for broadly reacting CK probes, while tumorous stroma expressed vimentin as we expected. The CK reactivity in canine SCC was complex and heterogeneous. In addition to CK5 and 14, both markers of stratified epithelia, CK8 staining (marker of simple epithelia) was noticed either focally or more generally. On the other hand, one or more CKs belonging to the following set was (were) revealed: CK4, 7, 10, 13, 18 and 19. So far, our data are in accordance with those obtained on human SCC (E. g. Moll et al., 1982, 1986) and fail to reveal a clear correlation between the CK composition and the degree of differentiation of SCC lesions.

The CK reactivity of CE again obligatorily included CK5 and in addition CK10, 14 and 19 in varying amounts, (i. e. mixture of «stratified» and «simple» CKs). This CK set corresponds to the CK composition observed in human keratoacanthoma where again CK4, 7, 13 and 18 were absent. (e. g. Moll et al., 1984, 1986). A pilomatricoma site, present in the mixed type of CE (neck, Table 1) repre-

sents a similar CK profile as in human pilomatricoma, i. e. it is reminiscent of the CK staining observed in the keratogenous zone of the hair bulb matrix cells differentiating into cortex cells (Broekaert et al. 1990).

Finally, the CK set of BCT once more is composed of «single» (CK 7, 8, 18, 19) and «stratisfied» CKs (CK 5, 10, 13, 14). Only CK5 (with all proper reserve), CK8 and CK14 were systematically expressed. The heterogeneity and the complexity of the CK composition observed are similar to that observed in human basal cell carcinomas (e. g. Moll et al., 1982, 1984, 1986). The recurrent absence of CK4 is the only negative constancy. As in human BCC, the presence of the phenotypic keratinization marker CK10 is also associated with foci of terminal differentiation in canine BCT.

Table 1: Survey of CK expression data in skin epithelial tumours (dog).

		Cytokeratin										
Localizatio	n Differenti	atic	n									
		4	5	7	8	10	13	14	16	18	19	BS
-SCC-												
Back	Good		+	-	+f	+f		+	+ -	-		+
Mandible	Good	—	+		+f	-	-	+	+ -	-	-	+
Toe	Moderate	+f	+	-	+f	+f	+f	+	ND-	_	-	+
Buttock	Bad	+f	(+)	+f	+	+	+f	+	ND:	+f	+f	+
Tongue	Bad	-	+	+f	+f	-	-	+	+ -	-	-	+
-CE-												
Neck	Good	_	(+)	_	+	+f	-	+f	ND-	-	+f	+
Forehead	Good		+	-		+f	-	+f	ND-	-	+f	+
-BCT-												
Neck	Garland	_	(+)	_	+	_	_	+f	ND-	-	_	+
Back	Garland	_	(+)	_	+	+	-	+	ND-	_	-	+
Mandible	Garland	_	(+)	_	+	_	-	+	+f -	-	-	+
Shoulder	adenoid	_	(+)	-	+	-	-	+f	ND-	-	-	+
Thorax	adenoid	_	(+)	+	+	+	f	+	ND-	+f	+f	+
Head	adenoid	_	(+)	_	+	_		+	+f -	_	_	+

f = focal expression; ND = not determin; (+) = CK5 possibly expressed in presence of CK8

BS = broad spectrum

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PATHOLOGY AND ETIOLOGY OF NATURAL AND EXPERIMENTAL EUROPEAN BROWN HARE SYNDROME

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In recent years an increasing mortality has been recorded in the brown hare (lepus europaeus P.) in various European countries (Gavier et al., 1988). In the majority of cases, the cause of death appeared to be liver failure due to a severe necrotizing hepatitis. This hepatic lesion seems to be a consistent finding, whereas lesions in other organs may be present in varying frequencies. considering that a histologic lesion was the only common characteristic, and considering also that the cause was unknown, this disease has been dessignated provisionally as «European Brown Hare Syndrome» (EBHS). Both toxins (e. g. 00 rapeseed) and infectious agents (e. g. Clostridium sordelli) have been suggested as possible etiological factors. Nevertheless there is growing evidence that 00 rapeseed, although an important cause of death in the roe, probably is not involved in the brown hare syndrome. Preliminary experiments point to a possible viral etiology (Eskens and Volmer, 1989). The striking resemblance of the liver lesions in EBHS and those described in Viral Haemorrhagic Disease of rabbits (VHD) has led some authors to suggest a common etiology (Marcato et al., 1989). Unfortunately in this disease also the etiology is still a matter of considerable controversy. Recently Morisse (pers. commun.) has succeeded in reproducing symptoms and lesions characteristic of VHD in rabbits by the oronasal and intramuscular route in 6 rabbits using an inoculum obtained from the livers of 2 hares. He considers that both VHD and EBHS are caused by a calici-like virus present in bile and liver homogenate. Nevertheless sofar no evidence has been produced of a direct association between a virus and the liver lesions in EBHS. In the present study the histologic lesions of both natural and experimental EBHS have been investigated in detail and ultrastructural evidence of a viral etiology is given.

Material and methods

Between september and december 1989, 62 hares have been autopsied, 44 of which were wild hares found dead or captured moribund. From the wild hares samples were taken systematically from the following organs for histological examination: liver, conjunctiva, cornea, retina, trachea, apical and diaphragmatic lung lobes. In selected cases samples also were taken from spleen, thymus, kidney, adrenals, cerebral cortex, cerebellum, brain stem, stomach fundus, duodenum, jejunum, ileum, caecum and colon. These samples were fixed in formalin and embedded in paraffin. Sections were made and stained with H&E or other staining techniques when indicated. For the experimental studies, 4 captive hares were obtained from an isolated farm, where no previous outbreaks of EBHS were recorded.

These animals were inoculated as described elsewhere (Nauwynck et al., in preparation). In short, a bacteria free suspension of conjunctivae, lungs and livers from wild and captive hares that died with lesions typical of EBHS, was used for the inoculations. At euthanasia (1 animal), or very shortly after natural death, samples for histological examination were taken as in the field cases. Samples for ultrastructural studies were taken from the liver, the trachea and the apical and diaphragmatic lung lobes. These samples were embedded in a Spurr-Epon mixture and contrasted with uranyl acetate and lead citrate. Samples of liver homogenate were examined by immuno electron microscopy following negative staining with potassium phosphotungstate.

Results

Out of the 44 wild hares, 25 were classified as EBHS on the basis of positive histopathological criteria and absence of other possible causes of death. The other group of 19 animals contained a variety of different diagnoses, in which rodentiosis and coccidiosis were important.

The gross lesions in animals classififed as EBHS were rather inconsistent. In the majority of cases icteric discoloration was seen of the sclera, the aorta intima, the subcutis and tendons. Internal organs in these animals usually were congested, but not haemorrhagic. The mucosa of the trachea in particular usually appeared dark red. Ten of these animals showed a catarrhal to necrotising conjunctivitis associated with an opacity and occasional ulceration of the cornea. Liver lesions usually were inconspicious. Only in some cases an increased marking and pinpoint central redening of the lobules was noted.

At histological examination however, the liver lesions were marked. These lesions were used to classify an animal as EBHS positive. In all cases there was an obvious necrosis of hepatocytes, which was either diffuse or limited to the cells located at the periphery of the lobules. Non necrotic cells were swollen and showed an increased eosinophilia of the cytoplasm. Infiltrating lymphocytes usually were present near Kiernan's triangle. In some cases fatty degeneration of hepatocytes was seen. Bile duct proliferation also was observed in the majority of cases. In contrast to the lesions described in aflatoxicosis, fibrosis was not observed in any of the examined livres of hares. None of the other organs showed consistent histologic alterations, except for the lungs, where apical cytoplasmatic droplets were seen pinching off from bronchiolar epithelial cells. The conjunctivitis lesions proved histologically to represent a variety of inflammatory lesions. In the cases where Actinobacillus was isolated on bacteriology, the lesions usually were frankly purulent.