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The size differed from less than one cm diameter to more than ten cm with a cauliflower-like appearence.

Histologically an epithelial structure with a varying degree of connective tissue is the dominating feature but tumors with a biophasic growth pattern consisting of sarcomatous and epithelial structures also occur. Generally there is a papilliform growth often associated with tubular to adenoid configurations which were seen in several cases, especially in the adult group. Tumor cells in the adult animals appeared mainly columnar or cubidal whereas in the calves the tumor cells had a cuboidal or often a polymorph appearance. They had vesicular nuclei with distinct to prominent eosinophilic nucleoli and especially in the polymorph cells a vacuolated cytoplasma. In the younger animals multinucleated tumor cell were frequent. Neutral and acid mucopolysaccharides in the lumen of the tubuli and intracytoplasmic were demonstrated histochemically by the PAS and PAS-diastase stain. Ferruginous bodies as seen in human mesotheliomas were not present. The tumor cells of one adult animal revealed ultrastructurally well differentiated epithelial cells with desmosomes and tight junctions, basally located nuclei, abundant mitochondria and elongated microvilli.

Discussion

Mesotheliomas in calves seem to be less well-differentiated than in adults. Especially the tubular tumor structure in the adult cow requires a differential diagnosis towards adenocarcinoma (Stünzi u. Engeli, 1958). PAS-positive substances still present after diastase digestion can not exclude mesotheliomas from consideration for the final diagnosis as it is recommended for differentiation of mesotheliomas and adenocarcinomas in humans (Antman, 1986). The appearence of elongated microvilli as seen in this material may be helpful in the diagnosis (Sutton, 1988). Though ferruginous bodies were not observed a connection to asbestos exposition as is discussed pathogenetically in humans and in bovine mesotheliomas cannot be excluded Croft, 1983).

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IMMUNOHISTOLOGICAL STUDIES ON RABBIT HAEMORRHAGIC DISEASE (RHD)

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RHD was first described 1984 in China. 1986 it appeared in Italy. 1988 outbreaks of RHD were observed in France, Germany, Switzerland, Eastern Europe, Spain and Portugal. 1989 the disease reached Mexico (1, 2, 3, 4, 6).

The mortality of RHD amounts about 98% in infected farms and only rabbits older than 2 months become ill.

The causative agent is supposed to be a virus: some authors isolated a Parvovirus (3, 5), closely related to Murine and Porcine Parvovirus, some others a Calicivirus or a Picornavirus (2, 4).

The diagnosis of RHD is based on clinical signs, histopathological findings in liver and kidney (1) as well as HA of liver homogenates. Furthermore the diagnosis can be verified serologically (haemagglutination inhibition test, enzyme linked immunosorbent assay) (2, 7). In the present study formalin-fixed paraffin-embedded tissue sections from rabbits were investigated for RHD using an ABC-Peroxidase method.

Material and methods

An ABC-Peroxidase method was performed on formalin-fexed and paraffin-embedded tissue as follows: the sections were first deparaffinized in xylene and alcohol, counterstained with haematoxylin for 1 minute and rinsed in tap water. The sections were then put in a methanol bath containing 3% H_2O_2 for 5 minutes and washed in phosphate buffered saline (PBS) pH 8.0 for 5 minutes. The nonspecific reactions were reduced by applying normal rabbit serum, diluted 1:40 in PBS, for 1 hour in a humid chamber at room temperature. This serum was then tapped off and the slides were incubated over night in a humid chamber at room temperature with biotinylated rabbit anti-RHD serum, diluted 1:50 in PBS. This serum was obtained from a rabbit out of a farm with RHD history which did not become ill (Institute for Viral Diseases and Immunoprophylaxis, Basel). The biotinylation was done using standard methods. The slides were then washed three times for 5 minutes in PBS. ABC (Vector Laboratories, Burlingame, California, USA) was prepared following the manufacturer's instructions and then applied on the sections and incubated for 30 minutes at 37C. After three washes, Amino-Ethyl-Carbazole (AEC) was used as substrate to reveal antigen-antibody reaction. The slides were then rinsed in tap water and mounted in glycerol-gelatine.

Tissues from 17 rabbits were included in this study. 10 of these had been diagnosed as RHD on the basis of HA and/or histology. 4 cases were suspected and 3 cases had no history or signs of RHD. Sections of liver and kidney from all the animals jas well as spleen, lymph nodes and lungs from some animals were tested.

To investigate the close relationship described between Porcine Parvovirus (PPV) and the RHD virus both blocking experiments and an ABC-Peroxidase method, similar to the RHD-ABC-Peroxidase method, were developed by the use of Swine-anti-PPV serum (Institut for Viral Diseases and Immunoprophylaxis, Basel).

Results

The results are summarized in Table I.

When tested for RHD, hepatocytes from infected rabbits showed intense intranuclear staining suggestive of inclusion bodies and

diffuse intracytoplasmic staining, mainly in the periportal areas. In some animals positive staining of macrophages in the lungs, the spleen and lymph nodes was observed. All the 10 animals in which RHD was diagnosed by histology and/or HA were recognized as positive in immunohistology. None of the suspected and negative cases showed positive reaction. The only disagreement was observed in case 6 which had a low HA-titer but was negative in immunostraining.

When used in blocking experiments, unconjugated swine-anti-PPV serum did not inhibit the specific staining for RHD.

When tested for PPV by means of PPV-ABC method, no intranuclear or intracytoplasmic staining was seen.

Discussion

A good correlation was found between the HA and the ABC-Peroxidase method: 10 cases were tested with these two methods: all the cases showed the same results in immunohistochemistry and in haemagglutination.

A good correlation was also seen between immunohistology and histology: all the 9 rabbits showing «typical histological lesions» turned out to be positive in immunohistology. In the 4 cases of histologically suspected RHD, the results of the ABC testing was negative. All of the cases without arguments for RHD stained negative. The disagreement in case 6 might be due to autolysis and the low quantity of detectable antigens on the tissue sections.

Gregg and House (3) found a close relationship between the RHD virus and the PPV. They observed an intranuclear staining when testing for RHD by immunohistochemistry. In our study, the reaction was present in the nuclei and in the cytoplasm. The intranuclear reaction could be well due to an infection with a Parvovirus-like agent which does not crossreact with PPV. However the cytoplasmic staining might be due to another virus, maybe a Calicivirus or a Picornavirus.

This ABC-Peroxidase method can be used to facilitate the diagnosis of RHD even in autolytic tissues and to study retrospectively formalin-fixed paraffin-embedded tissues.

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Table 1: Comparison between immunohistochemistry, HA and histology

Case nr.	Immunohistochemistry	HA-titer	Histology
1	++ (1)	nd	typical
2	negative	nd	suspected
3	++ (1)	nd	typical
4	++ (1)	nd	typical
5	negative	negative	suspected
6	negative	1:128	suspected (autolysis)
7	+ (1)	1:2048	typical
8	++ (1)(4)	1:2048	typical
9	++ (1)(2)(3)(4)	>1:2048	typical
10	+ (1)	1:2048	(autolysis)
11	negative	negative	not typical
12	+ (1)	1:512	typical
13	++ (1)	>1:8192	typical
14	++ (1)	1:4096	typical
15	negative	nd	not typical
16	negative	negative	not typical
17	negative	nd	suspected

immunostaining:
(1): intranuclear and intracytoplasmic staining in hepatocytes
(2): spleen with positive reacting macrophages
(3): lymph nodes with positive reacting macrophages
(4): lungs with positive reacting macrophages
nd: not done

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COMPARISON OF ADHESION OF ENTEROPATHOGENIC E. COLI (EPEC) IN VIVO AND IN VITRO: AN ULTRASTRUCTURAL STUDY

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Enteropathognic E. coli isolates in humans attach in two different ways to HEp2 and HeLa cells. In the diffuse adherence (DA) pattern the bacteria are seen on the whole surface of the HeLa cell. In the second, the bacteria bind to localized areas of the HeLa cells and form very clear cut microcolonies. This is called localized adherence (LA) (Scaletsky et al., 1984). In addition Nataro et al. (1987) discribed an aggregative adhesion pattern where bacteria adhere to the cells but also to the petri dish. With the help of a DNA probe, these authors (1987) identified an EPEC adherence factor (EAF) on 97% of EPEC with LA. This EAF is involved in the in vivo and in vitro adherence (Knutton, 1987; Tzipori, 1989).

The first aim if this study was to answer the question whether the LA to HeLa cells belongs to the attaching and effacing E. coli (AEEC) and if this pecularity to attach in vitro to HeLa cells corresponds with the in vivo attachment to intestinal epithelium of the calf. Secondly we were interested whether the ability to produce cytotoxin was an essential factor for the attachment or if strains that produced no cytotoxin could attach equally in vivo and in vitro. A third aim of this work was, to establish an in vitro system, that could ascertain the