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Autor: Sydler, T. / Pospischil, A. / Bertschinger, H.U.

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attaching and effacing E. coli which would eliminate the use of experimental animals.
Eight field strains of calves presumably EPEC, as well as a positive and a negative control were tested for their attaching and effacing ability in the intestinal loop test. The same strains were brought on HeLa cells to evaluate the adherence patterns, followed by an ultrastructural control of the attaching and effacing in vitro.
Two of 8 field strains and the positive control showed the typical attaching and effacing (AE) with cups and pedestals in the intestines of the calf. The lesions were the same in the loops of the ileum and in the distal jejunum. The strains showing AE in the gut showed a similar lesion on HeLa cells and made LA.
In later in vitro tests of two isolates that produced no shiga like toxin in Vercell tissue culture but made LA on HeLa cells, we used Pearson's (1989) AEEC strain as a positive control. The reference strain and one field strain adhered perfectly to the HeLa cells. The production of cytotoxin seems not to be necessary for AE. These results indicate that the formation of cups and pedestals on HeLa cells observed in the transmission electron microscope corresponds with the AE in vivo. The LA is useful as a screening test for AEEC, but the ultrastructural investigation is still required to demonstrate AE. Therefore HeLa cells are suitable for the identification of AEEC of the calf and make the use of experimental animals superfluous. However, further investigations are necessary to further elucidate the mechanism of attaching and effacing.

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Institut für Veterinärpathologie, Universität Zürich.

IMMUNOHISTOCHEMICAL DETECTION OF CHLAMYDIAE IN FORMALIN-FIXED TISSUE SECTIONS: COMPARISON OF A MONOCLONAL ANTIBODY WITH YOLK DERIVED ANTIBODIES (IGY)

U. Straumann-Kunz, A. Pospischil, M. F. Paccaud

Immunohistological detection of chlamydiae in formalin-fixed and paraffin-embedded sections of various organs from several species is described. In a retrospective study, two antisera, a commercially available monoclonal murine antibody (IgMur) and vitelline immunoglobulins (IgY), extracted from the egg yolk of immunized hens, were compared and tested for their applicability under routine condition. Both antisera were applied to tissues from which chlamydiae had been isolated or in which the presence of chlamydiae had been suspected in specially stained sections. Antigen labelling was optimal with the monoclonal antibody. Vitelline immunoglobulins produced some unspecific reactions, especially in lung tissue sections. Because of the antigenic relationship between the vitelline antibodies and tissues of birds, IgY are not suitable for the detection of psittacosis on avian substrates, when using an indirect immunological method. Staining in other tissues e. g. intestine or placenta was of equal quality as that attained with monoclonal antibodies. Depending on the advantages and disadvantages in every individual case one of the two antibodies may be chosen for further studies. Vitelline antibodies should be prefered with respect to animal welfare. (To be published in Journal of Veterinary Medicine Series B 1991).

Institutes of Vet. Pathology and Vet. Bacteriology, University of Zurich, Switzerland

PERIPHERAL INTESTINAL NERVOUS SYSTEM (PLEXUS SUBMUCOSUS) IN EXPERIMENTAL OEDEMA DISEASE OF CONVENTIONAL PIGS

T. Sydler, A. Pospischil, H. U. Bertschinger

Escherichia coli enterotoxaemia (edema disease = ED) is caused by only a few serotypes of *E. coli* (0 138:81B, 0 139:12B and 0 141:85B) and is regarded as a degenerative angiopathy leading to oedema in various tissues. The typical findings of a full stomach and a nearly empty small intestine raise the question whether there are significant morphological changes in the peripheral intestinal nervous system, possibly due to direct neurotoxic effects. We examined the ultrastructure of the Plexus submucosus (= Meissner) in experimentally infected pigs which were killed in the course of a study on intestinal colonisation by an enterotoxaemic *E. coli* strain.
Thirteen of 19 infected animals showed clinical ED-signs, of which at necropsy 12 had oedema in one or more characteristic location. The most common lesions in the plexus were axon swelling of slight to moderate degree, rarely accompanied by ruptures and autophagic vacuoles containing degenerating myelin-like products. In all animals with plexus lesions only some but not all plexus were altered and within the altered plexus the lesions were not homogenous. Plexus lesions were seen in infected pigs with clinical ED (9 of 13) as well as in infected pigs without ED (4 of 6) as well as in not inoculated pigs (6 of 11). A difference between the three groups could not be found, particularly since 6 of 19 infected pigs had no plexus lesions.
Nearly all animals had concurrent infections with at least one of the following infectious agents: rotavirus, chlamydia, cryptosporidia, Balantidium coli, ascarids and still not identified protozoa. Rotavirus was demonstrated in 12 pigs, however in 7 of the 11 pigs without plexus lesions no rota virus was found. It is not clear which direct or

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.

Dep. Vet. Pathology & Laboratory of Physiol. Chemistry, State University of Gent, Belgium.

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

Thoonen H., Broekaert D., Coucke P., Maenhout T., Hoorens J.

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) represents 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										
Localization	Differentiation	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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