Zeitschrift: Schweizer Archiv für Tierheilkunde SAT : die Fachzeitschrift für Tierärztinnen und Tierärzte = Archives Suisses de Médecine Vétérinaire ASMV : la revue professionnelle des vétérinaires

Herausgeber: Gesellschaft Schweizer Tierärztinnen und Tierärzte

Band: 132 (1990)

Heft: 8

Artikel: Comparison of adhesion of enteropathogenic E. Coli (EPEC) in vivo and in vitro : an ultrastructural study

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DOI: https://doi.org/10.5169/seals-593708

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

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

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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).

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

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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