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PEYER'S PATCHES AND THE FOLLICLE-ASSOCIATED EPITHELIUM IN DIARRHEIC CALVES*

PATHOMORPHOLOGY, MORPHOMETRY AND ACID PHOSPHATASE HISTOCHEMISTRY*

By

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LANDSVERK, T.: Peyer's patches and the follicle-associated epithelium in diarrheic calves. Pathomorphology, morphometry and acid phosphatase histochemistry. Acta vet. scand. 1981, 22, 459—471. — Twelve 2—5-week-old calves affected with a spontaneous intestinal disorder were examined; 8 had diarrhea and 4 were convalescents. In all the affected calves the "pseudovilli" (syn. domes or lymphoid villi) over Peyer's patches seemed atrophic and appeared enclosed within the mucosa, owing to fusion of ordinary villi with "pseudovilli". Morphometric examination showed a decrease of lymphoid follicle length in the affected calves as compared with controls (P < 0.01). Convalescents showed longer follicles than diarrheic calves (P < 0.05). Often cytoplasmic acid phosphatase of the follicle-associated epithelium (FAE) in affected calves did not show the marked basal-apical decrease along "pseudovillus", typical of the controls. Scanning electron microscopy revealed sparse development of concentric folds in the luminal plasma membrane of the enclosed FAE, contrasting with their abundance in the normal FAE. Transmission electron microscopy showed that the "pseudovilli" had increased numbers of ordinary villous epithelial cells. Affinity of chlamydia for FAE was shown. It is suggested that the sparse occurrence of surface folds in the FAE and the change in acid phosphatase distribution indicate diminished endocytosis of antigenic material, probably resulting from the enclosure of "pseudovilli". The atrophy of lymphoid follicles may be another expression of the probable decreased contact with the intestinal contents.

Peyer's patches; epithelium; microfolds; acid phosphatase; intestinal lesions; follicle atrophy; chlamydia; diarrhea; calves.

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In a previous study the follicle-associated epithelium (FAE) covering the "pseudovilli" (syn. domes or lymphoid villi) above Pever's patches in calves was described (Landsverk 1981a). This epithelium is apparently specialized for a pinocytic function. It has been thought that the FAE transfers antigens from the intestinal contents to immunocompetent intraepithelial lymphocytes, with subsequent blast cell formation in the underlying lymphoid follicles (Bockman & Cooper 1973, Owen 1977, Cebra et al. 1979). The sensitized blast cells migrate via the intestinal lymphatics and the thoracic duct to the blood and from the blood into the intestinal lamina propria, to form the IgA-secreting B-cell population (Craig & Cebra 1971, Husband & Gowans 1978, Husband et al. 1979). The "pseudovilli" of Peyer's patches thus appear to sample antigen from the gut, a function that also seems to make them especially exposed to intestinal infections (Gianella et al. 1973, Prescott et al. 1980).

Lesions of the "pseudovilli" were frequent among calves with spontaneous diarrhea (*Landsverk* 1981b). Because of the probable importance of these structures to the establishment of intestinal immunity, a further study of the lesions seemed warranted. This paper gives a detailed description and interpretation of the lesions and tries to evaluate their influence on lymphoid follicle development and their possible role in the pathogenesis of the disease.

MATERIALS AND METHODS

The material comprises 8 diarrheic calves (1-4, 7-10) and 4 convalescent calves (5, 6, 11, 12) also used in other studies (Landsverk 1981b, 1981c, Landsverk et al. in prep.). Seven healthy calves (13, 14, 17-21) served as controls. Calves 1-14 were given milk replacers with constituents including either normally treated (diet B1, calves 1-6; diet B3, calves 13, 14) or heat-damaged (diet B2, calves 7-12) skim milk powder and whey powder. Calves 17-21 were given whole cow's milk (WM). The calves with diarrhea were euthanized at different stages of the disease, when they were 2-5 weeks of age. Further details on the experiments and procedures are given in other reports (Landsverk 1981b, Landsverk et al. in prep.).

Morphometric examination was performed with an ocular micrometer. The length and width of follicles of Peyer's patches in the ileum were measured. The follicle length was measured from their base near the muscularis externa to the base of the crypts surrounding the "pseudovilli". The measurements were made for each calf on at least 6 properly oriented follicles (Figs. 1, 2) in Carnoy fixed sections, Statistical analyses of the morphometric data were made using the chi-square test. The chi-square results were confirmed by the analysis of variance employing "nested classifications" as a model.

The method for acid phosphatase (EC 3.1.3.2) histochemistry has been described earlier (*Landsverk* 1980). Incubations of sections from posterior jejunum 3 and ileum were performed. Controls were included.

For scanning electron microscopy (SEM) a modification of the tissue processing previously described was used. It proved difficult to get access to the surface of the "pseudovilli" of the diarrheic animals by using the previous techniques (*Landsverk* 1979), since the "pseudovilli" became enclosed in the mucosa during the disease. The technique of digital pressure fracturing, referred to by *Miyai* (1978), was therefore adopted. The manual rupture was performed on tissue fixed in a diluted Karnovsky's fixative (*Karnovsky* 1965) containing 0.9 % glutaraldehyde and 0.7 % paraformaldehyde in 0.14 mol/l cacodylate buffer. After the rupture the specimens were dehydrated and critical point dried as in the previous SEM procedure. The ileum was selected for transmission electron microscopy; the method has been described earlier (*Landsverk* 1981a).

RESULTS

A description of the outbreak and duration of diarrhea, microbiologic examination, macroscopic findings and parts of the microscopic examination of the gastrointestinal tract is given elsewhere (*Landsverk* 1981b, *Landsverk et al.* in prep.). For the purposes of the present report a brief statement of the disorder described in the other reports may be given: The diarrhea of calves 1—12 was mainly attributed to the occurrence of intestinal infections. Serologic examinations indicated the probability of a wide-spread rotavirus infection, and studies of frozen sections showed positive immunofluorescence for rotavirus in the intestinal villi of calves 1 and 8. Calf 1 showed in addition occurrence of large numbers of Gram negative bacteria, probably Escherichia coli, on the intestinal villi. Calf 9 had large numbers of pseudomonas in the ruminal and in the intestinal contents. Calves 3, 5, 11 and 12 were shown to be infected with chlamydia.

The light microscopic appearance of the "pseudovilli" of the controls has already been described (Landsverk 1979, 1981a) (see Figs. 1, 2), and the lesions of the "pseudovilli" in the affected calves 1-12 have been briefly reported elsewhere (Landsverk 1981b). In more detail, the changes in calves 1-12 were as follows: The "pseudovilli" appeared atrophied and often seemed to be squeezed between the thickened and shortened ordinary villi. Fusion between ordinary villi and "pseudovilli" was frequent (Figs. 3, 4). The epithelia had grown together in the lower as well as the apical portions of the "pseudovilli". In the fused areas the normal organization of epithelial cells was frequently disturbed, the cells being arranged to form a bridge structure (Fig. 4). In some fused areas contact between the lamina propria on both sides was established. As a result of these processes the free surface of the "pseudovilli" accessible for communication with the intestinal lumen appeared to be much reduced, the "pseudovilli" apparently being enclosed in the mucosa. Accumulations of neutrophils were frequent in the pockets formed between the "pseudovilli" and the ordinary villi (Fig. 5). The occurrence of chlamydia inclusions within the "pseudovillous" epithelial cells (calves 3, 5, 11, 12) has been described (Landsverk 1981b).

The results of the incubations for acid phosphatase are given in Table 1. Whereas there was a marked basal-apical decrease in the cytoplasmic acid phosphatase reaction in the epithelial cells along the "pseudovilli" of the controls (Fig. 6), affected calves showed a less pronounced gradient; in some "pseudovilli" there was no decrease at all (Fig. 7).

Scanning electron microscopy of the "pseudovilli" in the controls has been described (*Landsverk* 1979, 1981a). A special feature may be emphasized in this context: The "pseudovilli" protrude into the intestinal lumen (Figs. 8, 9), but their basal portion is inserted into depressions of the mucosa (Fig. 10). Digital pressure fracturing revealed the lymphoid follicles (Fig. 8,) which completely filled out the space between muscularis mucosae and tunica muscularis. The "pseudovilli" of the diarrheic and convalescent calves were apparently almost enclosed by and squeezed between the ordinary villi (Figs. 11—13), impressions

Calf No.	Diet	Reaction
1		+
2		+/++
3		+/++
4	B1	++/+++
5		++
6		++
7		++
8		+/++
9		+/++
10	B2	+
11		++
12		++
13, 14, 17—21 (Controls)	B3, WM	0/+

T a ble 1. Acid phosphatase reaction in follicle-associated epithelial cells of the upper $\frac{2}{3}$ of the "pseudovilli" in the posterior jejunum 3/ileum.

+++ =strong; ++ =moderate; + =weak;

0 =no reaction; / =variation of the reaction.

on the surface of the "pseudovilli" apparently being due to pressure from ordinary villi (Fig. 12). Ragged circumcript areas, without surface membrane structures, were frequently found on the "pseudovilli"; they were interpreted as fused areas that had been separated from the ordinary villus during the manual rupture of the tissue (Fig. 13). Generally, the ragged areas were oriented perpendicularly on the ruptured surface of the specimens. Bridge formations oriented at other angles between "pseudovilli" and ordinary villi were sometimes seen. Remnants of adjacent tissues occasionally adhered to the "pseudovilli" (Fig. 11). The plasma membrane of enclosed "pseudovillous" epithelial cells differed from the controls (Fig. 14) by having less developed longitudinal folds, their surface being constituted by regular although small microvilli (Fig. 15). In the vicinity of fused areas epithelial cells with longer and more densely crowded microvilli, resembling those of ordinary epithelial cells, were seen. Frequently the surface of the enclosed "pseudovillous" epithelial cells was covered by a filamentous material (Fig. 16).

Transmission electron microscopy showed a number of special features in the "pseudovillous" epithelium of the calves 1—12, as compared with healthy calves (Landsverk 1981a). Fusion between the ordinary villous epithelium and the "pseudovillous" epithelium was frequent (Figs. 17, 18, 20). In the fused areas the two epithelia had developed intercellular connections and also cytoplasmic prolongations which seemed to interdigitate. In proximity to the fused areas the "pseudovilli" were sometimes populated by groups of epithelial cells resembling ordinary absorptive epithelial cells (Fig. 21). These cells showed a development of microvilli with longitudinal microfilaments and an absence of endocytic vesicles and vacuoles. Controls occasionally had single cells of this type which were interposed between the other typical "pseudovillous" epithelial cells. The epithelial cells adjacent to the fused areas occasionally had microvilli with extra long microfilaments (Fig. 20).

Accumulations of neutrophils and profiles of a membraneous material were found in the spaces between the "pseudovilli" and the ordinary villi (Figs. 18-20); the membraneous material was probably derived from goblet cell secretions (Fig. 18). The material appeared to be internalized by the "pseudovillous" epithelial cells and accumulated in multivesicular bodies (Fig. 19). Accumulation of this membraneous material possibly caused the extreme electron opacity of the multivesicular bodies (Figs. 17, 19). Dilatation of the basal portion of the intercellular spaces was regularly seen in the controls; this feature was not prominent in the diarrheic calves. Groups of apical "pseudovillous" epithelial cells sometimes showed evidence of degeneration; they were electron opaque and had many autophagic vacuoles, and seemed to be extruded. The enclosed "pseudovilli" seemed to have fewer intraepithelial lymphocytes than the controls. Globule leukocytes were markedly reduced, whereas the number of neutrophils varied, sometimes being accumulated in large numbers.

In calves containing chlamydia-like inclusions as seen by light microscopy, micro-organisms with morphology corresponding to that described for chlamydia were found in the "pseudovillous" epithelial cells (Fig. 22), but only occasionally in the adjacent ordinary epithelium (*Landsverk* 1981b). The chlamydia were in different developmental stages and were enclosed within membrane bound vacuoles. Dense bodies were occasionally seen in proximity to the chlamydia. Varying degree of cell degeneration with swollen mitochondria, dilated endoplasmic reticu-

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F i g u r e 1. Mucosa with Peyer's patches, control calf (17). Principal regions are: "Pseudovilli" (P), ordinary villi, lymphoid follicles (F), and interfollicular areas (I). HE, \times 40.

Figure 2. Mucosa with Peyer's patches, diarrheic calf (7). The crypts are hyperplastic. A "pseudovillus" (P) and atrophied follicles (F) are seen. HE, \times 40.

Figure 3. Mucosa over Peyer's patches, diarrheic calf (2). The ordinary villi are atrophied and crypts are hyperplastic. The "pseudo-villi" (P) appear to be enclosed within the mucosa. Arrows indicate fusions between the "pseudovilli" and the adjacent epithelium. HE, \times 60.

Figure 4. Convalescent calf (11). Several areas of fusion between the "pseudovillus" (P) and the adjacent epithelium. HE, $\times 250$.

Figure 5. Convalescent calf (11). Aggregation of neutrophils in a "pocket" formed between the "pseudovillus" (P) and the adjacent tissue. HE, \times 120.

F i g u r e 6. Acid phosphatase, control calf (20). The epithelial cells in the apical portion of the "pseudovillus" (P) contain almost no reaction product, whereas the basal portion of the epithelium, subepithelial macrophages and adjacent ordinary epithelium react strongly. No counterstain. \times 110.

F i g u r e 7. Acid phosphatase, convalescent calf (11). The apical cytoplasm of the "pseudovillous" (P) epithelial cells is relatively strongly stained. The subepithelial macrophages and adjacent ordinary epithelium are likewise strongly positive. Arrow indicates fused area. No counterstain. \times 110.

F i g u r e 8. Scanning electron micrograph, control calf (21), digital pressure fracturing. The Peyer's patches consist of lymphoid follicles (F) which tend to fill out the space between muscularis mucosae and muscularis externa. Arrow indicates a "pseudovillus". \times 40.

Figure 9. Scanning electron micrograph, control calf (18). The "pseudovillus" (P) is localized at the base of ordinary villi (V). The surface of the "pseudovillous" epithelial cells protrudes and shows occasional pores (arrows). \times 550.

F i g u r e 10. Scanning electron micrograph, control calf (17). The specimen was fractured accidentally after critical point drying, leaving basal portions of the mucosa open for inspection. The "pseudovilli" (P), some of which are fractured, are inserted into depressions of the mucosa, whereas fractured ordinary villi (V) are inserted at a higher level. \times 90.

F i g u r e 11. Scanning electron micrograph, diarrheic calf, digital pressure fracturing. The "pseudovillus" (P) is partially obscured by adhesive tissues, probably owing to fusion with adjacent epithelium. \times 120.

F i g u r e 12. Scanning electron micrograph, diarrheic calf (9), digital pressure fracturing. The "pseudovillus" (P) has apparently been squeezed between the ordinary villi, leaving an impression on the "pseudovillus'. \times 400. F i g u r e 13. Scanning electron micrograph, diarrheic calf (8), digital pressure fracturing. The "pseudovillus" (P) with apex indicated (A) has irregular ragged areas with loss of surface membrane structure, interpreted as ruptured areas, probably formed during the manual rupture of the tissue (arrows). The apical portion of the "pseudovillus" is apparently intact. \times 800.

Figure 14. Scanning electron micrograph, control calf (17). The surface of the "pseudovillous" epithelial cells has central microvilli and peripheral folds, the direction of the folds following the course of the cell borders. \times 5,000.

F i g u r e 15. Scanning electron micrograph, diarrheic calf (9), digital pressure fracturing, enclosed "pseudovillus". The epithelial cell surface has closely spaced microvilli, peripheral folds are almost absent. \times 4,000.

F i g u r e 16. Scanning electron micrograph, diarrheic calf (8), digital pressure fracturing, enclosed "pseudovillus". The surface of the "pseudovillous" epithelial cells is covered by a filamentous material. \times 2,000.

F i g u r e 17. Diarrheic calf (4). Fusion between epithelial cells of the "pseudovillus" (to the left) and ordinary villus (to the right). Notice the different electron opacity of the two epithelia and the lack of longitudinal microfilaments in the sparse microvilli of the "pseudovillous" epithelial cells. The "pseudovillous" epithelial cells show numerous dense multivesicular bodies (arrows). L = intraepithelial lymphocyte. $\times 8,000$.

F i g u r e 18. Diarrheic calf (4). Fusion between the epithelial cells of the "pseudovillus" (lower portion) and the ordinary villus (upper portion). A goblet cell (G) seems to empty its contents into the space formed between the two epithelia where numerous membraneous profiles are seen. \times 11,000.

F i g u r e 1 9. Diarrheic calf (7). Apical portion of a "pseudovillous" epithelial cell. Membraneous profiles are found in the intestinal lumen. The electron opacity of the contents in endocytic vesicles and tubules and a multivesicular body (MvB) may have resulted from pinocytosis of the membraneous material. \times 30,000.

F i g u r e 2 0. Diarrheic calf (4). Fused area between "pseudovillous" epithelial cells (P) and ordinary villous epithelial cells (V). Intercellular connections are developed between the two epithelia. Accumulation of membraneous profiles in the lumen. Note the long micro-filaments in the microvilli and apical portion of an apparent ordinary villous epithelial cell (arrow). \times 18,000.

F i g u r e 21. Diarrheic calf (4). The "pseudovillus" is in part populated by cells resembling ordinary absorbing cells, i.e. showing lack of endocytic vesicles and having microvilli with microfilaments. An apparent cell fragment (F) in the lumen might have been formed by ecdysis. Accumulation of glycogen-like granules (Gl) in an epithelial cell. N = neutrophil. \times 6,000.

Figure 22. Diarrheic calf (3). Chlamydial organisms within vacuoles of "pseudovillous" epithelial cells. RB – reticulate bodies. EB = elementary bodies. Arrows point to electron opaque bodies, probably multivesicular bodies. N = neutrophil. \times 8,500.













lum, and fragmented plasma membrane were observed in the chlamydia infected cells. In calf 2 the "pseudovillous" epithelium showed marked degenerative traits with swollen mitochondria, dilatation and vesiculation of the endoplasmic reticulum, and occurrence of large heterogeneous dense bodies. Viral inclusions were not seen in any of the ileal specimens.

The results of the morphometric examination of the lymphoid follicles of Peyer's patches are given in Fig. 23. In the diarrheic and convalescent calves the lymphoid follicles showed a marked reduction of length (P < 0.01), whereas the width did not appear to be reduced. The convalescent calves also showed longer follicles than the diarrheic calves (P < 0.05).



Figure 23. Morphometric examination of the lymphoid follicles in ileum. The standard deviations are indicated.

DISCUSSION

The present diarrheic and convalescent calves consistently showed lesions of the "pseudovilli" over Peyer's patches, the most outstanding feature being fusion of "pseudovilli" with ordinary villi. The high frequency of fusions associated with the "pseudovilli" is in contrast to the low frequency of fusions between ordinary villi, and indicates probably that the "pseudovilli" are especially prone to this anomaly.

The cellular processes resulting in fusion of epithelia are not clear. It seems possible, however, that damage to and extrusion of epithelial cells in apposed villi are followed by stretching and fusion of the remaining cells in an effort to cover the denuded areas. Immobilization of the apposed villi is probably of some importance in this process, since the "pseudovilli", with their accumulation of lymphoid cells in a loosely woven supporting tissue, are likely to be more immobile than ordinary villi. It is well known that a continuous "pumping" movement occurs in ordinary villi. No smooth muscle cells were seen in preliminary studies of the "pseudovillous" core (Landsverk, unpublished). It may also be of some significance that the "pseudovilli" in healthy calves are inserted in depressions of the mucosa, a condition which may cause immobilization through increased contact with the adjacent mucosa. This condition might have been further advanced by the elongation of crypts (Landsverk 1981b) that accompanied the apparent "pseudovillous" atrophy. The increased occurrence of ordinary absorbing cells along the "pseudovilli" in proximity to the fused areas may have been created by transfer of ordinary absorbing epithelial cells during the process of fusion. The epithelial cells are continuously migrating from crypt bottoms to villous tips (MacDonald et al. 1964), and migration from one villus to another in areas where lamina propria of both sides have contact, does not seem impossible.

The normal function of the follicle-associated epithelium (FAE), covering the "pseudovillus", appears to be the sampling of antigens for immunological purposes (*Cebra et al.* 1979). It may be that specialization of the tissue for this sampling function makes the FAE especially susceptible to infectious agents. A number of infections seem to have a predilection for Peyer's patches (*Sprinz et al.* 1956, *Maenza et al.* 1970, *Carter & Collins*

1974, Hohmann et al. 1978). The actual preferential microbial invasion of the FAE has been described (LaBrec & Formal 1961, Gianella et al. 1973, Prescott et al. 1980), corresponding to the predilection of chlamydia for the "pseudovillous" epithelium in the present study.

The affinity of micro-organisms for FAE is apparently not restricted to virulence characteristics. Thus innocuous Escherichia coli (Hohmann et al. 1978) and even 2 μ m latex particles (Le Fevre et al. 1978) are taken up in Peyer's patches. It therefore seems probable that the FAE is not only capable of pinocytosis but also of phagocytosis of larger particles. The subsequent intracellular handling of micro-organisms in the FAE may favor survival. Lysosomes probably have some protective function in the ordinary absorbing epithelium. Fusion of invading bacteria and epithelial lysosomes seems to occur in salmonellosis (Takeuchi 1967). The upper two-thirds of the "pseudovillous" epithelium shows a weak acid phosphatase reaction, indicating low lysosomal capacity (Landsverk 1981a), although this trait was not so marked in diarrheic as in healthy calves.

The fusion between the "pseudovilli" and adjacent epithelium caused an apparent enclosure of the "pseudovilli". The actual extent of the enclosure and reduction of contact between the "pseudovilli" and the intestinal contents is not known. As judged from the altered morphology and functional state of the FAE, it seems probable, however, that this contact was markedly reduced. Prominent folds are characteristic of the surface of FAE in healthy calves and may be interpreted as a specialization for bulk transport (Landsverk 1981a). The low occurrence of such folds in the enclosed FAE may be an expression of decreased endocytosis, caused by lack of material available for uptake. The mere absence of surface folds does not imply abolished pinocytosis, as invaginations of the surface membrane were frequent and seemed to occur independently of fold formation. Rather it may appear that fold formation is related to special forms of bulk transport, perhaps to the uptake of larger particles. The marked decrease of acid phosphatase in the epithelial cells from the base of the "pseudovilli" to the more apical portions typical of healthy calves, may be related to an altered functional state of the cells, i.e. to the onset of endocytosis (Landsverk 1981a). Thus, the less marked decrease in affected calves may be another expression of diminished endocytosis.

The apparent atrophy of the lymphoid follicles under the "pseudovilli" may possibly be another expression of diminished uptake of antigenic material by the FAE. It has been suggested that the FAE transfers antigens to the intraepithelial lymphocytes by a process comprising pinocytosis and reverse pinocytosis (Owen 1977). The sensitization of lymphocytes is probably followed by an enhanced blast cell formation in the lymphoid follicle (Waksman et al. 1973). It appears that the maintenance of follicle size may be dependent on contact with contents of the intestinal lumen. Studies on the significance of this contact have been made on the rabbit appendix, which contains lymphoid structures similar to Peyer's patches (Waksman et al.). When the rabbit appendix is surgically obliterated and its contents removed, its lymphoid follicles show atrophy (Blythman & Waksman 1973). Neonatal rabbits subjected to this procedure show decreased follicle development in the appendix (Perey & Good 1968, Stramignoni et al. 1969). Furthermore, in gnotobiotic animals the development of Peyer's patches is seriously retarded (Thorbecke 1959, Cooper et al. 1968, Pollard & Sharon 1970). Peyer's patches are known to atrophy in early adult life (Reynolds 1980), although part of the lymphoid tissue remains and is probably responsible for the response to antigens present in the intestinal lumen, a function that is maintained throughout life.

A direct influence of micro-organisms must also be considered as a possible cause of the follicle reductions. Necrotic areas in the Peyer's patches lymphoid follicles were found in experimental chlamydia infection of neonatal calves (*Doughri et al.* 1974). Although chlamydia were also found in some of the present calves, necroses in the lymphoid follicles were not seen.

In conclusion, it may be suggested that the follicle reduction and the decreased occurrence of surface folds of the FAE were related to diminished contact with the intestinal contents and, probably, decreased antigen transfer to the lymphoid tissue. The present calves may thus have had a compromised capacity for antigen detection and immune response, a condition that may have influenced the duration and severity of the intestinal disorder.

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SAMMENDRAG

Peyerplettene og det follikkel-assosierte epitelet hos diarékalver.

Peverplettene og det follikkel-assosierte epitelet (FAE) ble undersøkt hos tolv 2-5 uker gamle kalver; 8 hadde diaré og 4 var rekonvalesenter. Kalvene hadde atrofiske "pseudovilli" (syn. lymfoide villi) som så ut til å være lukket inne i mukosa p.g.a. sammenvoksninger mellom "pseudovilli" og ordinære villi. Morfometrisk undersøkelse viste lavere follikkelhøyde hos de affiserte kalvene (alle 12), enn hos kontrollene (P < 0.01) og likeså lavere follikkelhøyde hos diarékalvene enn hos rekonvalesentene (P < 0.05). Hos affiserte kalver var det ofte ikke så markert basal-apikal nedgang av sur fosfatasereaksjon i det follikkel-assosierte epitelet (FAE) langs "pseudovillus" som hos kontroller. Ved skanning elektron mikroskopi viste FAE hos affiserte kalver sparsom forekomst av folder i overflaten, strukturer som det vanligvis er rikelig av i det normale FAE. Transmisjonselektronmikroskopi viste at "pseudovilli" hos de affiserte kalvene i forøket grad var bekledt av ordinære absorberende epitelceller. Chlamvdier viste en utpreget predileksjon for FAE. Det antydes at den sparsomme utviklingen av folder og forandringene i sur fosfatase reaksjon i FAE kan ha sammenheng med nedsatt endocyttose. Sammen med atrofien av "pseudovilli" og follikler kan dette være uttrykk for en mangelfull antigentilførsel fra tarminnholdet, forårsaket av innelukkingen av "pseudovilli".

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