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## Review article

# MALT structure and function in farm animals

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**Abstract** – Mucosa-associated lymphoid tissue (MALT) is defined as an organized lymphoid tissue in the mucosa that samples antigens. The morphological characteristics that distinguish MALT from lymphoid infiltrates are discussed. MALT has been extensively investigated in laboratory animals, while knowledge in cattle, sheep, goats, pigs and horses that are summarized under the term farm animals in this review is fragmentary. Literature data about the distribution, morphology, function and involvement in infectious diseases of MALT in farm animals are described. The understanding of specific features of MALT in other species than laboratory animals is important for comparative research, in order to understand pathological and immunological processes in the respective species and as a potential route of vaccination of mucosal surfaces.

## MALT / NALT / tonsils / BALT / GALT

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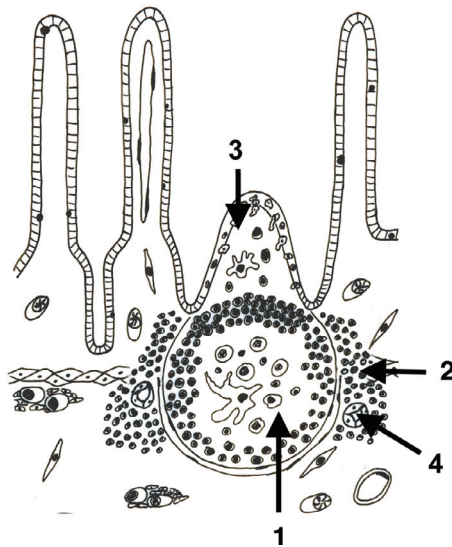
## 1. INTRODUCTION

Organized mucosa-associated lymphoid tissue (MALT) which is widely distributed in mucosal surfaces is an essential part of the mucosal immune system. MALT is the initial inductive site for mucosal immunity: antigens are sampled from mucosal surfaces and cognate naïve B- and T-lymphocytes stimulated. MALT structures are the origin of lymphocyte trafficking to mucosal effector sites. MALT contains lymphatics which transport immune cells and antigens to regional lymph nodes that can therefore be called part of the inductive

sites of mucosa and augment the immune responses.

MALT is located at strategical sites to allow efficient antigen sampling from mucosal surfaces. Based on the anatomical localization, MALT structures can be subdivided into [17]:

- conjunctiva-associated lymphoid tissue (CALT),
- lacrimal drainage-associated lymphoid tissue (LADLT),
- salivary gland-associated lymphoid tissue/duct-associated lymphoid tissue (SALT/DALT),



**Figure 1.** The morphological characteristics of MALT are the compartmental organization in lymphoid follicles with a light zone, dark zone and mantle zone (1), interfollicular areas (2) with HEV (4) and domes (3) with LE.

- nose or nasopharynx-associated lymphoid tissue (NALT),
- lymphoid tissues of Waldeyer's ring,
- larynx-associated lymphoid tissue (LTALT),
- bronchus-associated lymphoid tissue (BALT),
- gastric mucosa-associated lymphoid tissue (gastric MALT),
- gut-associated lymphoid tissue (GALT).

Only lymphoid tissues in the mucosa that fulfill certain morphological and functional criteria should be called MALT [17].

The structural characteristics of MALT are the following (Fig. 1):

1. Lymphoid tissue is located in close contact to the mucosal surface.
2. It consists of organized, structured lymphoid tissue with lymphoid follicles and T cell-dependent interfollicular areas. Lymphoid follicles (primary and with germinal

centers) are predominantly formed by B lymphocytes embedded in a network of follicular dendritic cells (FDC), and smaller numbers of CD4<sup>+</sup> T-lymphocytes and macrophages. Interfollicular areas contain predominantly CD4<sup>+</sup> and CD8<sup>+</sup> T lymphocytes.

3. MALT may occur as single (isolated) lymphoid nodules (ILF) or as larger aggregates of several lymphoid nodules.

4. The epithelium overlying MALT is severely infiltrated by lymphocytes and thus termed lymphoepithelium (LE). LE may contain morphologically distinct cells specialized in the uptake of antigens. They are named M cells based on their morphology in Peyer's patches. "M" is the abbreviation for "membranous" cell, since the apical cytoplasm of these cells is often reduced to a thin membrane, or "microfold bearing" cell, since microfolds have been described in M cells of non-human primates.

5. Antigen sampling occurs through the LE by M cells or dendritic cells. There are no afferent lymphatics.

6. Some components of MALT are constitutively present at defined mucosal sites, such as the tonsils; others are constitutively present, but their location varies, such as Peyer's patches in the jejunum, and others are induced by antigen exposure, such as BALT and ILF.

7. Recirculating lymphocytes enter MALT through high endothelial venules (HEV) in the interfollicular areas. Distinct receptors on HEV regulate the tissue-specific immigration of lymphocytes.

These characteristics help to distinguish MALT structures from focal lymphocytic infiltrates of the mucosa which have no compartmental organization, consist predominantly of T lymphocytes, and very importantly have no direct contact with the epithelium and thus the luminal antigen. The fate of these infiltrates is not resolved, since consecutive samples are difficult to obtain. It has been suggested that they may either resolve or develop into ILF. Focal infiltrates may be induced by infections and

**Table I.** MALT structures described in cattle, sheep/goats, pigs and horses.

	CALT	NALT	Waldeyer's ring		DALT LDALT	LTALT	BALT	Gastric MALT	GALT
			Nasopharynx	Oropharynx					
Bovine	+	nd	T. pharyngea T. tubaria	T. lingualis T. veli palat. T. palatina	nd	+	(+)	nd	+
Ovine / caprine	+	+	T. pharyngea T. tubaria	T. lingualis T. veli palat. T. palatina	nd	+	(+)	nd	+
Porcine	+	nd	T. pharyngea T. tubaria	T. lingualis T. veli palat.	nd	+	(+)	+	+
Equine	+	+	T. pharyngea T. tubaria	T. lingualis T. veli palat. T. palatina	nd	+	(+)	nd	+

+: constitutively present; nd: not examined / not described; (+): not constitutively present.

careful examination is necessary to distinguish for example ILF in the large intestine of pigs from granulomas induced by *Oesophagostomum dentatum* [39] or BALT from pneumonic lesions [6] or gastric MALT from gastric lesions [34].

MALT has been intensely investigated in laboratory rodents, since MALT plays a mayor role in the protection of mucosal barriers and also in allergic reactions. Investigations involving human tissues are limited to biopsy or resection material. Therefore data obtained in laboratory rodents are often extrapolated to humans, although mice, rats and rabbits are quite distinct in development, lifespan, environment (germ free, SPF), nutrition, and physiology from humans, and other species such as farm animals might be better as animal models.

The distribution, occurrence, morphology, ontogeny and evolution of MALT vary between species. In the following the current knowledge will be summarized for cattle, sheep, goats, pigs and horses which were arbitrarily combined under the term "farm animals" to distinguish them from the commonly used small laboratory animal species. Differences between species might be caused by anatomical and physiological characteristics, since MALT structures are always strategically located at

sentinel positions for optimal antigen sampling. The appearance of MALT is also influenced by differences in antigen exposure, e.g. due to management practices, since its development is often antigen-driven. Knowledge about MALT in farm animals is important for the following reasons:

- to recognize the normal structures to understand the physiology,
- to localize MALT, e.g. to collect it for diagnostic tests [121],
- to distinguish normal MALT from hyperreactive, activated, altered MALT [6, 34, 72],
- to learn about the pathogenesis of infections/host reactions [104, 105, 114, 117],
- to determine if inductive sites for the local application of vaccines are present [7, 59, 116],
- to investigate MALT structure and function under comparative aspects [37].

MALT has not been described at all sites initially listed in each of the farm animal species which does, however, not imply that it does not exist. The current knowledge of the distribution of MALT in cattle, sheep, goats, pigs and horses is summarized in Table I.

## 2. CONJUNCTIVA-ASSOCIATED LYMPHOID TISSUE (CALT)

CALT was investigated in a comparative study for cattle, sheep and pigs [24]. In these species, CALT is formed by variable numbers of ILF which are predominantly localized along the palpebral surface of the forniceal conjunctiva [24]. In goats, ILF and aggregated lymphoid nodules are present in the upper, lower and third eyelid [7]. CALT has been described in humans, but not in mice or rats [24].

Primary and secondary lymphoid follicles were found in the lymphoid nodules of CALT with secondary lymphoid follicles more common in sections with higher numbers of lymphoid follicles [24]. Lymphoid nodules are covered by areas of LE, but morphologically distinct M cells were not identified [24]. Some of the flattened epithelial cells in the LE of goats show preferential uptake of ferritin and are functionally comparable to M cells [7]. The potential of the conjunctiva to internalize bacteria was demonstrated for *Salmonella abortusovis* in lambs [113]. Infection which was locally restricted or systematically propagated depending on the dose of bacteria was induced in lambs by conjunctival inoculation. Thus CALT should be included as a potential site for the application of vaccines [113].

In sheep, CALT has increasingly received interest for diagnosis of scrapie, since it is a site where peripheral lymphoid tissue can be easily harvested in live animals without clinical side effects. Comparative sampling from the third eyelid, tonsil and mandibular lymph node has revealed the highest yield of lymphoid follicles in biopsies of the third eyelid [121].

## 3. NOSE-ASSOCIATED LYMPHOID TISSUE (NALT)

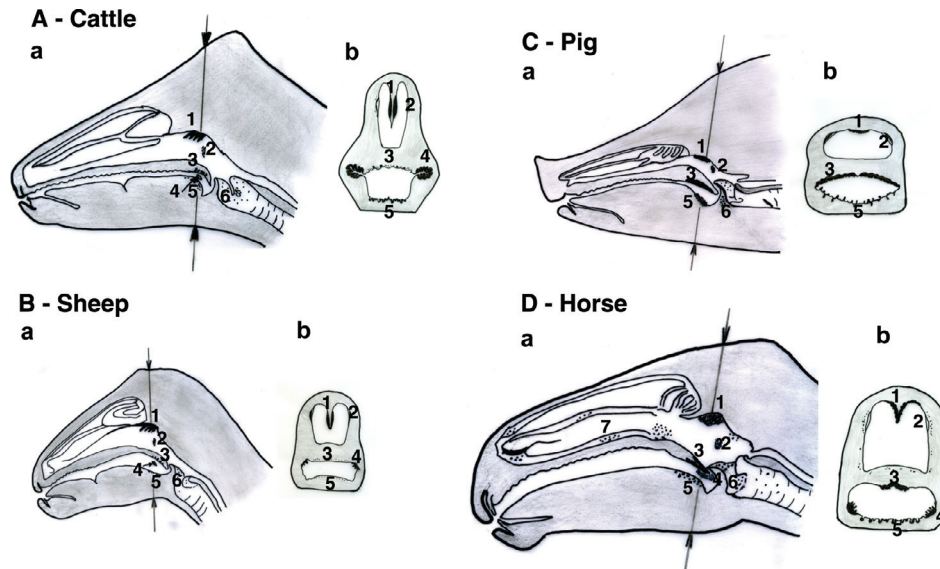
NALT is well described and characterized in small laboratory animals [55, 119, 132]. It is present as paired lymphoid aggregates in the floor of the nasal cavity at the

entrance to the pharyngeal duct [55]. In farm animals, no comparable aggregates are found at these sites, but ILF have been described in horses and sheep [76, 77, 120]. ILF are most likely also present in the nasal mucosa of other farm animal species, but have not been investigated yet. A practical reason for this may be that the nasal mucosa is difficult to dissect from the nasal cavity and the preparation for histology involves decalcification steps which further inhibit immunohistochemical characterization of the immune cells.

In sheep and horses, NALT was made visible by acetic acid treatment [77, 120]. In sheep, ILF with well developed germinal centres and LE with M cells were concentrated posterior to the opening of the Eustachian tube [120]. In the horse, ILF were found consistently at defined sites in the nasal cavity: in the nasal vestibule, the middle and ventral meatus, the caudal ventral conchae and the nasopharyngeal walls ([77], Fig. 2D). ILF were already present in 10 to 11 month old fetuses and neonates. There was a marked increase in the number of ILF in young adult horses and an age related decrease in horses of more than 10 years. Lymphoid nodules in the anterior nasopharynx were present beneath polypoid epithelial protruberances, those in the caudal nasopharynx were associated with crypts [76]. The amount of lymphoid tissue in the nasal cavity was smaller than in the lymphoid tissues of Waldeyer's ring [77].

## 4. LYMPHOID TISSUES OF THE WALDEYER'S RING

In contrast to small laboratory rodents, the lymphoid tissues of Waldeyer's ring are well developed in farm animals and humans [55]. Therefore interest has centered on them instead of NALT. Lymphoid tissues of Waldeyer's ring guard the nasal, oral and auditory passages into the pharynx. They are formed by large aggregates of lymphoid nodules termed tonsils that occur constitutively at distinct anatomical sites in the



**Figure 2.** Distribution of lymphoid tissues of Waldeyer's ring (1–pharyngeal tonsil, 2–tubal tonsil, 3–tonsil of the soft palate, 4–entrance to palatine tonsil, 5–lingual tonsil), LTALT (6) and NALT (7) in cattle (A), sheep (B), pig (C) and horse (D), median (a) and transversal section (b, plane indicated), modified from [118].

pharynx of each species and ILF that vary in number (Fig. 2). In the oropharynx, the lingual tonsil, the palatine tonsil and the tonsil of the soft palate are present; in the nasopharynx, the pharyngeal and tubal tonsils are present.

Tonsils are important for inducing immunity at mucosal sites. Some pathogens have, however, developed mechanisms to overcome tonsillar defenses and may use them as the port of entry, replication and colonization [48]. Several pathogens are able to persist asymptotically within the tonsils making the identification of carriers difficult in disease control and elimination. Therefore tonsils are highly important tissues for diagnostic investigations of infectious diseases.

The pharyngeal and tubal tonsils are the main targets for nasal vaccines which are attractive, because of the relative accessibility and high permeability of tonsils and the microenvironmental conditions with

less acidic pH, lower levels of enzymatic activity and no ruminal digestion [120]. Although not widely used, nasal vaccination may provide a practical alternative to oral vaccination to induce mucosal immune responses. The intranasal inoculation of sheep with *Pasteurella hemolytica* caused a significant increase in the size of BALT, a significant increase in numbers of BALT structures and had a protective effect against colonization [32, 135].

#### 4.1. Bovine

##### 4.1.1. Composition of the Waldeyer's ring

In the bovine, a moderate amount of lymphoid tissue is present in Waldeyer's ring ([78, 118], Fig. 2A). The oropharynx is protected by the lingual tonsil, the palatine tonsil and the tonsil of the soft palate. The lingual tonsil consists of many cryptolymphatic units (CLU) which extend from the root of the tongue along the lateral pharynx

to the epiglottis. CLU are epithelial crypts which are surrounded by one or more lymphoid follicles and interfollicular areas. The palatine tonsils are global masses of lymphoid tissue embedded in the submucosa of the lateral wall of the oropharynx. In the pharynx, only the openings where the epithelial crypts enter the lymphoid tissue are visible. The tonsil of the soft palate consists of a few CLU in the oral aspect of the soft palate. The pharyngeal tonsils are formed by two ridges of lymphoid tissue not penetrated by epithelial crypts in the membranous part of the nasal septum. The tubal tonsils are found within the pharyngeal openings of the auditory tube. The pharyngeal tonsil and the palatine tonsils have been examined in detail because they account for the major part of the lymphoid tissue of Waldeyer's ring in the bovine.

#### **4.1.2. Pharyngeal tonsils**

The pharyngeal tonsils are initially formed by 8 to 14 parallel rugae separated by furrows which develop at 95 days of gestation [116]. Primary lymphoid follicles occur at 5 months of gestation. They increase in number and size in the late fetal and postnatal period. Patches of microvillus-bearing cells interpreted to be M cells are found in the ciliated epithelium at 5 to 6 months of gestation. The pharyngeal tonsil is not fully developed at birth, but differentiates after antigen contact [116]. The typical compartmental organization and the development of germinal centers and LE develops 2 to 4 weeks after birth [116]. The low number of germinal centers and intraepithelial lymphocytes in calves may contribute to their increased susceptibility to infections [116]. The size of the pharyngeal tonsils decreases in animals over 7 years of age. Especially the size of lymphoid follicles is reduced and there is a relative increase in the proportion of T lymphocytes [116].

#### **4.1.3. Palatine tonsils**

The palatine tonsils are also not fully developed at birth, but differentiate further

after antigen contact [78]. In 3 week old calves, the palatine tonsils are still unorganized, but they are well organized in 2 month old calves [78]. In adult cattle, numerous secondary lymphoid follicles with distinct light, dark and mantle zones are distinguishable [126]. Within germinal centers follicular dendritic cells were identified by transmission electron microscopy [126].

#### **4.1.4. Differences between tonsils and other MALT structures**

There are subtle differences between the different tonsils and other MALT structures as far as homing and recirculation of lymphocytes are concerned [96]. PNA<sub>d</sub> is expressed in a high percentage of HEV in the pharyngeal and palatine tonsil and much less in HEV of Peyer's patches, whereas MADCAM-1 is expressed in few HEV of the palatine tonsil, an intermediate percentage of HEV in the pharyngeal tonsil and a high percentage of HEV in Peyer's patches. Since PNA<sub>d</sub> binds especially to CD62L on naïve T-helper cells, their proportion is higher in tonsils than in Peyer's patches. MADCAM-1, in contrast, binds preferentially to B7 memory T helper cells and thus their proportion is higher in Peyer's patches than in tonsils.

#### **4.1.5. Role of tonsils in infection**

Bovine tonsils have been investigated in the context of several infectious diseases. The tonsils are infected early in the course of Bovine Herpes Virus 1 (BHV1)-infection. Viral antigen is present in the tonsillar epithelium and lymphoid tissue and causes necrosis/apoptosis of the tonsillar epithelium and lymphoid tissue in neonates, calves and adult cows [81, 117, 128]. Tonsils are also important for diagnosing asymptomatic carriers of BHV1, since the virus becomes latent in the lymphoid tissue of the tonsil and can be reactivated by immunosuppression causing renewed shedding [94, 129]. In Bovine Virus Diarrhea Virus (BVDV) infection, the tonsils are the initial site of infection in acute postnatal



infection [74]. In Foot and Mouth Disease (FMD), another important reportable disease of cattle, the virus persists and is shed for extended time periods (> 28 days) from tonsils, while it is cleared much earlier from epithelial sites and blood [136]. *Mycobacterium bovis* was cultured from tonsils with and without lesions in about 50% of tuberculin reactor cattle [20]. The palatine tonsils are more frequently affected than the pharyngeal tonsil indicating oral uptake of the pathogen. Tonsils have been shown to be reservoirs for *Mannheimia hemolytica* [33]. Infected calves can shed the pathogen from the tonsils for several weeks and can harbor it for long periods without shedding. In asymptomatic carriers, shedding can be induced by BHV1 infection [33].

The examples above demonstrate that tonsils are important in the pathogenesis of several infectious diseases of cattle some of which are even reportable. Tonsils are not only important tissues to diagnose overt diseases, but also to detect clinically inapparent carriers.

## 4.2. Ovine/caprine

### 4.2.1. Composition of the Waldeyer's ring

The lymphoid tissues of Waldeyer's ring in sheep and goats are even less developed than in cattle (Fig. 2B). Its main constituents are the pharyngeal tonsil, a ridge at the caudal part of the membranous nasal septum, the palatine tonsils formed by 3 to 6 fossulae with crypts in the same location as the bovine palatine tonsils and the tubal tonsils at the pharyngeal opening of the auditory tube [118]. The lingual tonsil consists of a small amount of diffuse lymphoid infiltrates at the dorsal root of the tongue. A few CLU in the oral mucosa underneath the tongue in goats form the sublingual tonsil. The tonsil of the soft palate consists of a small amount of diffuse lymphoid tissue.

The typical organization of MALT with distinct lymphoid compartments, lymphoid follicles with germinal centers and LE has

been described for the pharyngeal, palatine and tubal tonsils only [22]. In addition, dense aggregates of lymphoid tissue occur in all of the above sites and scattered lymphoid cells are universally present [22]. Tonsils reach maximal size in the first two years of life and regress afterwards.

### 4.2.2. Pharyngeal tonsils

A more detailed investigation of the surface of the pharyngeal tonsils has revealed that the ridges of lymphatic tissue are crossed by deep furrows. There are patchy areas with non-ciliated, microvillous bearing cells that are ultrastructurally similar to M cells at other mucosal sites and show a preferential uptake of carbon particles [22, 23].

### 4.2.3. Tubal tonsils

The tubal tonsils are composed of individual lymphoid nodules with lymphoid follicles containing B lymphocytes and FDC, parafollicular areas containing CD4<sup>+</sup>, CD8<sup>+</sup> and  $\gamma\delta$  T lymphocytes and dome-like accumulation of lymphocytes [120]. LE with a mixture of ciliated and microvillous bearing cells interpreted to be M cells occurs in the center of the epithelium above domes [120].

### 4.2.4. Palatine tonsils

The sequential formation of LE with M cells was investigated in the palatine tonsils of 1 to 21 day old sheep [83]. Initially M cell precursors and a local MHCII expression were observed in the epithelium. This was followed by the immigration of MHCII<sup>+</sup> dendritic cells into the epithelium and finally the immigration of lymphocytes forming the LE.

### 4.2.5. Role of tonsils in infection

Tonsils are important in several infectious diseases of sheep and goats. They are a port of entry for *Chlamydia psittaci* inducing seroconversion without infection of the

genital tract [53]. Apparently healthy sheep may harbor pathogens like *Salmonella* sp. and *Pasteurella hemolytica* in their tonsils [5, 46]. Tonsils have gained increasing interest over the last decade, since testing for scrapie was intensified and PrPsc accumulation has been reported in lymphoid tissues including tonsils early, in preclinical disease [115, 124]. Thus testing of tonsillar tissue allows preclinical screening for scrapie in healthy sheep and live-animal confirmation in suspect cases of scrapie [84].

### 4.3. Porcine

#### 4.3.1. Composition of the Waldeyer's ring

Lymphoid tissues of the Waldeyer's ring are well developed in swine ([48, 118], Fig. 2C). In the nasopharynx, the pharyngeal tonsil is present as a patch on the median roof of the nasopharynx and the tubal tonsils that form patches at the pharyngeal opening of the auditory tube. Epithelial crypts extend into these tonsils. In the oropharynx, there are two symmetrical, very large patches of lymphoid tissue with epithelial crypts on the ventral part of the soft palate. They form the tonsils of the soft palate, but are sometimes referred to as palatine tonsils which are missing in the porcine species. The lingual tonsil is well developed and consists of lymphoid nodules that accumulate in villous-like epithelial papillae and a few CLU. The tonsils have often been included when the porcine immune system has been investigated. Flow cytometry has demonstrated that tonsils are particularly rich in B lymphocytes, have moderate numbers of  $\alpha\beta$ -T lymphocytes and low numbers of CD8<sup>+</sup> and  $\gamma\delta$  T lymphocytes compared to lymph nodes, PBL and spleen [133].

#### 4.3.2. Tonsils of the soft palate

The tonsils of the soft palate consist of lymphoid follicles with B-lymphocytes and scattered CD4<sup>+</sup> and CD8<sup>+</sup> T lymphocytes and interfollicular areas with CD4<sup>+</sup> and CD8<sup>+</sup> T lymphocytes [54]. Multiple epithe-

lial crypts extend into the tonsil and branch extensively. Patches of LE characterized by high numbers of intraepithelial lymphocytes within the layer of non-keratinized epithelial cells, M cells and goblet cells are present within crypts [13]. The frequency and size of LE varies among and within animals [111]. The intraepithelial lymphocytes in the LE are B and T lymphocytes. Within the T lymphocyte subset, CD4<sup>+</sup> T lymphocytes are the most frequent followed by  $\gamma\delta$  and CD8<sup>+</sup> T lymphocytes [111]. M cells are not uniform, but have variable surface morphology [13]. The LE is active in the uptake of macromolecules [127]. The lymph leaves the tonsil through parafollicular sinuses that drain into efferent lymphatics [12].

#### 4.3.3. Lingual tonsil

Immature lymphoid follicles are observed in the lingual tonsil at day 77 of gestation [58]. They increase in size, but a development of germinal centers was not observed neither in germfree nor in conventional pigs [57].

#### 4.3.4. Differences between tonsils and other MALT structures

Tonsils of the soft palate and pharyngeal tonsil differ in the expression of vascular addressins and epithelial cytokines [18]. VCAM-1 is expressed on HEV of the pharyngeal tonsil, but not in the tonsil of the soft palate. In the pharyngeal tonsil high levels of the epithelial cytokine CCL28 and low levels of CCL25 were found. In contrast, the levels of CCL28 are very low in the tonsils of the soft palate. Differential expression of adhesion molecules in the different tonsils were detected both in pigs and cattle [18, 97]. Unfortunately different addressins were investigated in the studies, thus not allowing a comparison between the species. The differential expression of adhesion molecules may contribute to the heterogeneity of lymphocyte homing described in pigs (for review see [87]).

#### 4.3.5. Role of tonsils in infection

Many pathogens target porcine tonsils. Classical swine fever virus is detected in the tonsils early during infection [125]. In pigs naturally infected with pseudorabies virus, virus latency was demonstrated in the tonsils [107]. The earliest infection and replication of FMD virus occurs in the pharynx including the tonsils [4]. Pigs congenitally infected with porcine respiratory and reproductive syndrome virus can support virus replication for extended periods in the tonsils and lymph nodes [110]. Although replication occurs at low levels, virus is easily transmitted to sentinel pigs [110]. Lesions in postweaning multisystemic wasting syndrome which is associated with porcine circovirus 2 infection, affect lymphoid organs including tonsils [108]. Tonsillar carriers have been described for *Mycoplasma hyosynoviae*, *Streptococcus suis*, *Salmonella* sp. and *Yersinia pseudotuberculosis* [40, 82, 112, 130]. Therefore tonsils are very important tissues for diagnostic tests.

### 4.4. Equine

#### 4.4.1. Composition of the Waldeyer's ring

The lymphoid tissues of Waldeyer's ring are particularly well developed in the horse ([118], Fig. 2D). The lingual tonsil is formed by a collection of CLU at the root of the tongue. The tonsil of the soft palate forms an about 4 by 2.5 cm ridge on the oral aspect of the soft palate. The palatine tonsils extend as two symmetrical, 10 to 12 cm long and 2 cm wide ridges in the lateral pharynx from the basis of the epiglottis to the tongue. The pharyngeal tonsils are present in the dorsal nasopharynx at the end of the nasal septum as CLU. Diffuse lymphoid tissue and ILF around the pharyngeal openings of the auditory tube are called tubal tonsils.

The pharyngeal tonsils and the lingual tonsil have been investigated in detail.

#### 4.4.2. Pharyngeal tonsils

In 1 to 2 year old, healthy horses the pharyngeal tonsils consist of organized lymphoid nodules in the lamina propria [59]. Superficial folds and deep indentations of the epithelium reach into the lymphoid tissue. The pseudostratified, columnar, ciliated epithelium of the nasopharynx is multifocally replaced by patches of LE characterized by severe infiltrates of lymphocytes, the absence of goblet cells and the presence of non-ciliated, microvillus-bearing cells [59]. Some of these cells have been identified as M cells based on their ultrastructural features, positive reaction for vimentin and a distinct pattern of lectin binding [62].

#### 4.4.3. Lingual tonsil

The lingual tonsil was investigated for its possible role in oral infections, e.g. *Streptococcus equi* [60, 61]. Several layers of well organized lymphoid tissue are found in the CLU. Variably sized patches of LE were found in the crypts. Although no M cells were identified based on morphological characteristics, it is suggested that the LE of the lingual tonsil represents the functional counterpart to the LE of the pharyngeal tonsil [60, 61].

#### 4.4.4. Role of tonsils in infection

Equine tonsils may serve as reservoirs for pathogens, e.g. *Streptococcus zooepidemicus*. They are, however, most likely the inductive site to protect the upper respiratory tract against infections like influenza and *Streptococcus* sp. and are thus interesting targets for vaccines [62, 132].

### 5. LARYNX- AND TRACHEA-ASSOCIATED LYMPHOID TISSUE (LTALT)

Larynx-associated lymphoid tissue has been described on the epiglottis, in the

vestibulum laryngis and on the plica aryepiglottica of cattle, sheep, pigs and horses [118]. CLU found in two deep furrows at the base of the epiglottis of pigs and sheep form the tonsilla paraepiglottica. Accumulations of lymphoid nodules on the processus vocales of cattle are termed tonsilla glottica. In horses, lymphoid nodules at the laryngeal inlet are already present in nine month old fetuses, a marked increase of size is seen in neonates and young adult horses and an age-related reduction in horses over ten years [77]. In the trachea, lymphoid nodules have been described in horses only [76]. They occur in horses over two years and decline in number from rostral to mid trachea. Lymphoid nodules in the larynx and trachea of the horse are located beneath a polypoid protuberance of the surface epithelium and have the typical organization of MALT [76]. In addition infiltrates of unorganized lymphoid tissue occur.

## 6. BRONCHUS-ASSOCIATED-LYMPHOID TISSUE (BALT)

In contrast to the tonsils of Waldeyer's ring and GALT, BALT is not present before birth in cattle, sheep, goats, pigs and horses [6, 10, 77]. BALT is not constitutively present in farm animals and humans, but is very dynamic in these species [45, 88]. It has been suggested that this is typical in species with a well developed Waldeyer's ring, while species with little lymphatic tissue at Waldeyer's ring, like mice, rats or rabbits, have constitutively large amounts of BALT [55]. The occurrence and development in farm animals is antigen dependent and it may be severely enlarged in certain respiratory tract infections which cause so-called cuffing pneumonia indicating the potential for local inflammation to induce lymphoid tissue in airways. BALT structures are strategically placed in the lungs at sites where they are optimally impacted by inhaled antigens.

BALT is important in farm animals, since respiratory tract infections are a common and economically important problem. So far, methods for immunization against this disease complex, which often has multifactorial etiology, have not been uniformly successful. It is recognized that respiratory immunity is best correlated with local immune responses and the lymphoid tissue within the lung contributes to these responses. Therefore knowledge about BALT should help to develop methods to stimulate local respiratory immunity.

### 6.1. Bovine

BALT is not found in neonatal calves, increasing numbers of lymphoid nodules and lymphoid aggregates are seen in calves from 4 months to 18 months of age and there is an age-related reduction in numbers in older cattle [6]. BALT is more frequently present in cranial than in caudal lung lobes. Although the term bronchus-associated is used, it occurs without preference at all airway levels. Lymphoid tissue is located under the epithelium of larger bronchi, in the submucosa of small bronchi and extending from the epithelium to adventitia in bronchioles. In bronchioles, it is frequently located adjacent to an arteriole. Both organized lymphoid nodules with primary and secondary lymphoid follicles and unorganized aggregates of lymphocytes can be seen. They may be different developmental stages of the same structures [6, 35].

In calves with morphologic signs of enzootic pneumonia, lymphoid nodules increase from about 3 to 20 [6]. They are predominantly associated with bronchioles and regionally increased in the cranial lobes.

LE with non-ciliated epithelial cells was identified in pneumonic calves only [6]. Uptake of ink by non ciliated epithelial cells and macrophages in the LE was observed, however, in 2 to 8 week-old clinically healthy calves [42]. It is unclear, if these animals had histological signs of preceding

pneumonia, or if areas of active LE were more accurately detected by the tracer. Thus problems with identifying M cells in BALT may be due to variations of morphology and labeling patterns in the different compartments of the mucosal immune system.

### 6.2. Ovine/caprine

BALT is not present in neonatal goats, appears in 50% of 1 month old goats and markedly increases between 1 month and 1 year [10]. No lymphoid nodules, but only dense aggregates of lymphoid cells were identified in healthy sheep from 6 months to 9 years around bronchi and bronchioles, more frequently in small bronchi and bronchioles, predominantly below the muscularis [22]. They did not have compartmental organization or specialized LE.

BALT is more organized in antigen-challenged ovine lungs [52, 135]. More and larger BALT structures were described in goats with not specified pneumonia, while in chronic pneumonia only the number of lymphoid nodules was increased [10]. BALT may acquire the typical features of MALT in sheep and goats with lung infections [104, 105]. Hyperplastic BALT resulting in cuffing pneumonia has been recognized in mycoplasma infections for a long time. In goats, *Mycoplasma agalactiae* and *Mycoplasma bovis* which cause moderate bronchointerstitial pneumonia without macroscopic lesions, and *Mycoplasma mycoides* sp. and *Mycoplasma capricola* which cause marked pulmonary consolidation induce hyperplasia of BALT [104, 105]. Hyperplastic BALT is highly organized with secondary lymphoid follicles containing increased numbers of IgG<sup>+</sup> B lymphocytes and aggregates of CD4<sup>+</sup> T-lymphocytes. There is an overall increase of T-lymphocytes due to an increase of CD4<sup>+</sup> T lymphocytes, and an increase of macrophages and dendritic cells [105]. The interpretation, if these BALT structures are beneficial or should be considered as lesions is unclear. They might be important

to control the spreading of *Mycoplasma* sp. in the lung and to the blood, although this local immune reaction does not prevent clinical disease. On the contrary, lymphoid hyperplasia may contribute to disease by compressing small airways.

### 6.3. Porcine

Data about incidence vary from 33% in healthy pigs [89], 80 to 100% in 4 month old SPF pigs [28] and 100% in conventionally raised 11 to 13 week-old crossbred pigs [49]. There is no BALT in germ free pigs [51]. BALT is formed by single lymphoid follicles without distinct compartments that bulge into the airways. Eighty-two percent are located on bronchioles, 10% on respiratory bronchi and 8% on bronchi. LE is present, but there are no distinct M cells [49].

A significant increase of BALT structures per lung area was observed after infection with *Actinobacillus pleuropneumoniae*, especially if the infection had been preceded by oral immunization [28, 86]. BALT might have been the entry site for live or attenuated *Actinobacillus pleuropneumoniae* applied as a vaccine in aerosol form which protected the pigs from a considerable dose of these bacteria in an exposure [44]. After infection of pigs with *Mycoplasma hyopneumoniae*, hyperplasia of BALT is the most significant change. It results in the development of highly organized BALT structures as described in goats [114]. The activation of lymphoid tissue is most likely due to the release of proinflammatory and immunoregulatory cytokines induced by the infection [106].

### 6.4. Equine

The respiratory tract of horses is particularly at risk for infection, since large air volumes pass through the respiratory tract of the horse (100 000 L per 24 h in an adult horse) [29] and thus large amounts of inert and infectious particles may be carried

into the lung depending upon housing and environmental conditions. Therefore several surveys of the immune system of the equine respiratory tract were done [16, 47, 76, 77].

BALT is not present in fetuses and neonatal foals, but develops antigen-dependent in older horses [16, 77]. In adult horses from 2 to 16 years of different breeds, BALT was detected in 7 of 20 horses, with an individually varying frequency of lymphoid nodules [77]. Unorganized infiltrates of closely packed lymphocytes predominated and only a few organized lymphoid nodules were seen in small intrapulmonary bronchi [76]. In thoroughbreds, BALT formed by organized lymphoid nodules and unorganized lymphoid aggregates is well developed in 12 week-old foals. Reduced numbers are found at 1 year of age and they are mostly absent at 2 years of age [16]. Differences in the amount and activity of BALT have been discussed as a cause for the increased frequency of infections in young horses [8, 16]. Dysfunctions of the immune system may contribute to the chronic inflammatory processes in horses, such as heaves, recurrent airway obstruction and COPD.

## 7. GASTRIC-MALT

Gastric MALT in farm animals is unique in pigs. Individual lymphoid nodules are present in the submucosa and lamina propria of the lesser curvature of the gastric cardia and of the cardiac fundic diverticulum [34]. Gastric MALT was initially discussed as evidence of gastritis in experimental *Helicobacter pylori* infection in pigs [56]. The following studies in non-infected, healthy pigs revealed that gastric MALT develops in fetal pigs and is present at birth like the other MALT structures of the gastrointestinal tract [31]. In piglets, they are found as small inactive discrete homogeneous encapsulated aggregates of lymphocytes deep in the submucosa [34]. Activation of gastric MALT can be induced by colonization of piglets with *Helico-*

*bacter pylori*, but not by enteric bacterial or viral infections [34]. Large activated gastric MALT consisting of several lymphoid follicles with germinal centers can be found in sows exposed to a microbe-rich environment. The gastric epithelium is devoid of parietal and goblet cells in these areas and releases deep crypts with areas of LE between the lymphoid follicles. Gastric MALT nodules resemble lymphoglandular complexes described in the colon of pigs [80]. Since gastric MALT has been described particularly after *Helicobacter pylori* infection in humans, the porcine stomach is of interest for comparative investigations.

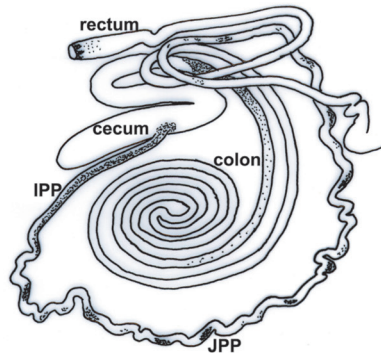
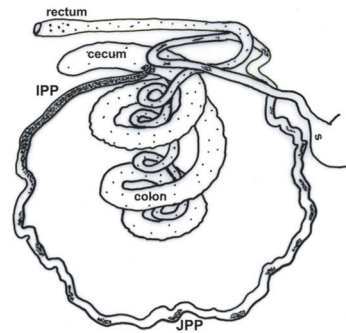
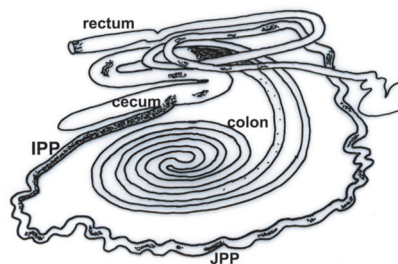
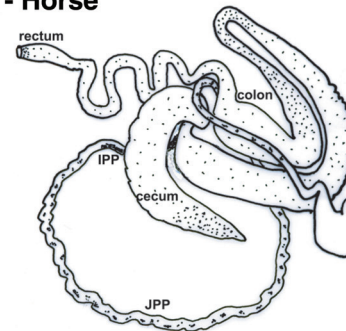
## 8. GUT-ASSOCIATED LYMPHOID TISSUE (GALT)

GALT is the part of the MALT that has been the best investigated, since it is an important entry site for antigens and infectious agents. Numerous studies were conducted to describe its distribution (Fig. 3) and to investigate the uptake of soluble and particulate material or infectious agents, despite difficulties using large animals in these experiments. LE of GALT may be the specific target of pathogens or may become infected as part of the mucosal surface. Infections may cause severe macroscopic lesions in GALT (fibrinous to erosive to ulcerative enteritis, button ulcers). Alterations of GALT do not cause localized lesions only, but affect the defenses of the entire gastrointestinal barrier, since reduced numbers of plasma cell precursors and primed T lymphocytes are produced. In sheep, GALT in the ileum was intensely investigated as a potential bursal equivalent [37].

### 8.1. Bovine

#### 8.1.1. Distribution, ontogeny and amount

GALT is present in cattle as patches in the jejunum (JPP), one patch in the ileum (IPP), a patch in the colon adjacent to the

**A - Cattle****C - Pig****B - Sheep****D - Horse**

**Figure 3.** Distribution of Peyer's patches and ILF in the small and large intestine of cattle (A) sheep (B), pig (C) and horse (D) modified from [118].

ileocecal opening, a patch in the proximal loop of the ascending colon, several small patches in the rectum along the anal ring and ILF in the small and large intestine (Fig. 3A).

Bovine GALT already develops in the fetus [19, 30]. JPP can be recognized in 5, the IPP in 6 to 7, and colonic lymphoid tissue in 6 month old fetuses. The number of JPP increases during fetal life, with up to 76 JPP in late term fetuses [30]. Carlens [19] reports between 24 to 49 JPP that develop in the fetus and remain for the entire life. Especially the IPP grows markedly during the fetal and neonatal period reaching up to 3 m of length. In contrast to all other GALT structures, IPP undergoes an age-dependent involution and is replaced by a few ILF in animals over two years [19].

On average 8.6% of the small and 7.8% of the large intestine are covered by PP in three month old calves [69]<sup>1</sup>. JPP of different sizes, spread irregularly in an antimesenteric position along the entire small intestine contribute about one third and the IPP about two thirds of GALT in the small intestine. In the large intestine, the major part is formed by the patch in the proximal colon which extends circularly and is 8 to 30 cm long. At the end of the patch there is

<sup>1</sup>Liebler E., Untersuchungen zur Anzahl, Verteilung und Ausdehnung der schleimhaut-eigenen Solitär-follikel und Peyerschen Platten im Dünndarm des Kalbes unter besonderer Berücksichtigung ihrer Oberfläche, Vet. Med. Thesis, Veterinary School Hanover, 1985.

a continuous change to ILF which can be found over the next 2 m of large intestine.

### 8.1.2. Histology and lymphocyte subsets

GALT in the different parts of the intestine has the typical organization of MALT structures, but there are distinct regional differences [66]. JPP have small pear-shaped lymphoid follicles and large interfollicular areas and domes. The IPP has long oval lymphoid follicles and small interfollicular areas and domes. With increasing age, lymphoid follicles with epithelial crypts occur particularly in the IPP [41]. In the large intestine, lymphoid nodules are found in the lamina propria or form lymphoglandular complexes (LGC) [69]. LGC are characterized by one or more lymphoid follicles in the submucosa and one or more epithelial crypts extending from the mucosal surface into lymphoid tissue [69].

Lymphocyte subpopulations do not differ between JPP and the IPP in fetal intestine [134]. In newborn calves differences become evident. In JPP and LGC, IgG- and IgA-mRNA expression and many T lymphocytes (predominantly CD4<sup>+</sup>) are present within lymphoid follicles; in the IPP fewer CD4<sup>+</sup> and CD8<sup>+</sup> T lymphocytes occur in lymphoid follicles and interfollicular areas [72, 92, 133]. Germinal centers develop only in JPP and colonic lymphoid tissue, but not in the IPP [134]. In adult cows, the IPP disappears and lymphoid follicles and interfollicular of JPP and LGC are larger than in calves. Flow cytometry revealed a marked increase of T-lymphocytes especially CD4<sup>+</sup> T lymphocytes and a decrease of  $\gamma\delta$  T-lymphocytes in JPP from cows [92].

### 8.1.3. Morphology and function of the lymphoepithelium

LE and M cells have been identified in all sites with GALT in the bovine. More enteroabsorptive cells than M cells are present in the LE of JPP and LGC, whereas the LE of the IPP is composed of an almost uniform population of M cells [63, 66, 93,

122]. One characteristic of M cells is the irregular short thick microvilli. Differences in microvillus development on M cells which are particularly obvious in LE in the large intestine were interpreted as different stages of cellular maturation [70, 71]. Multiple intraepithelial cells invaginate into M cells from the basolateral side causing the "membranous" appearance. Intraepithelial lymphocytes in the LE are predominantly B-lymphocytes.

Preferential uptake of ferritin was shown for M cells in all sites of GALT confirming the function of M cells in GALT [65, 71, 85]. There are regional differences in the efficiency of M cells to internalize material. Less ferritin was found in the M cells of the large compared to the small intestine and less in LGC compared to lymphoid nodules in the lamina propria [85]. Latex beads and parapox virus are internalized by M cells in the IPP, but not in JPP [65]. These differences have to be considered when oral vaccines are developed.

M cells serve as the port of entrance for pathogens, such as *Brucella abortus* [1] and *Mycobacterium paratuberculosis* [79]. Several infectious agents, such as astrovirus [131], bredavirus [95], rotavirus [96, 123], *Chlamydia* [63] and *Cryptosporidium* sp. [64], have been demonstrated in the LE of the small intestine. Severe lesions of GALT occur in mucosal disease and salmonella infection [9].

## 8.2. Ovine/caprine

### 8.2.1. Distribution, ontogeny and amount

GALT is present in sheep and goats as JPP and IPP in the small and ILF and patches in the large intestine at the ileocecal entrance, in the proximal colon, at the beginning of the spiral part of the ascending colon (2 to 12 cm long) and in the rectum around the anal ring (Fig. 3B).

Initially GALT develops independently of antigen exposure [102]. JPP may be detected in 60 day old fetuses, the IPP in



110 day old fetuses and patches in the large intestine in 90 day old fetuses [3, 101]. At birth 25 to 40 JPP are found and one IPP which may reach a length of 3 m in 2 month old lambs [19]. Vigorous lymphopoiesis occurs prenatally and reaches a maximum in 2 to 3 month old lambs [100]. Thereafter the IPP involutes and disappears at about 15 month. The JPP remain intact throughout life. Development of GALT in the large intestine resembles that of JPP: there is a postnatal expansion and a partial atrophy with age [3].

The number of lymphoid follicles was calculated to be about 100 000 in the IPP and 10 000 in the JPP [101]. Thus 90% of the GALT is located in the ileum in young sheep.

### 8.2.2. Histology and lymphocyte subsets

The same morphological differences between JPP and IPP as in cattle occur in sheep [101]. ILF in the large intestine are located in the submucosa and have dome-like structures with LE devoid of goblet cells [3]. Lymphocyte extravasation is much higher in JPP than in the IPP, where the majority of lymphocytes undergo apoptosis instead of entering recirculation [90, 103].

Cellular subsets in JPP and IPP are similar at birth, but diverge soon afterwards [38]. Lymphoid follicles of the JPP and colonic patches contain about 35 to 45% sIgM<sup>+</sup> B lymphocytes, 10 to 15% of CD4<sup>+</sup> T lymphocytes and 4 to 6% plasma cells [2, 36, 43, 67]. The percentage of  $\gamma\delta$  T lymphocytes is markedly higher only in the interfollicular areas of the rectal patches [2]. There is a high frequency of isotype switching. The IPP contains 95% sIgM<sup>+</sup> B lymphocytes and less than 1% CD4<sup>+</sup> T lymphocytes [36, 43, 67]. More lymphocytes expressing MHCII are present in the IPP compared to JPP [43]. During involution, an increasing number of lymphoid follicles resembling those of JPP can be found in the IPP [68].

### 8.2.3. The IPP as a primary lymphoid organ

The distinct morphology, as well as growth and involution characteristics of the IPP initiated discussion about its function as a primary lymphoid organ. The highly specialized microenvironment in the IPP promotes a high level of B lymphocyte proliferation and antigen-independent hypermutation of immunoglobulin genes [98, 99]. Thus it was concluded that the IPP is a primary source of sIgM<sup>+</sup> B lymphocytes and generates a pre-immune Ig repertoire, whereas the function of the JPP and colonic patches is mucosal immunity [37].

## 8.3. Porcine

### 8.3.1. Distribution, ontogeny and amount

GALT is present as JPP and IPP in the small intestine and patches and ILF in the large intestine (Fig. 3C).

Focal lymphoid infiltrates are found in the lamina propria of the jejunum as early as day 50 of gestation and lymphoid follicles at about day 90 to 100 of gestation [21, 58]. Eighteen Peyer's patches were counted on average in fetal pigs from day 95 to birth [21]<sup>2</sup>. The number of JPP was followed from neonates to adult animals [19, 27, 50, 90]<sup>2</sup>. It varied between individuals from about 20 and 30 JPP. When the distribution of JPP was compared in the same animals at 2 and 12 months of age, it became evident that the location of the individual JPP was constant [109].

A rapid growth of all Peyer's patches occurs during the last 10 days before birth and in the first weeks after birth [50]<sup>2</sup>. The markedly reduced growth in gnotobiotic pigs suggests that antigenic stimulation is

<sup>2</sup> Sahlender H.-T., Untersuchungen zur Anzahl, Größe, Verteilung und Morphologie der Peyerschen Platten im Dünndarm und der Solitärfollikel im Dickdarm bei Schweinefeten und neugeborenen Ferkeln, Vet. Med. Thesis, Veterinary School Hanover, 1989.

essential for the postnatal development of JPP [11, 57, 91]. Growth of the IPP occurs more rapidly, since it expands by increase in number and size of lymphoid follicles, whereas only the size of lymphoid follicles increases in JPP [91]. An age-related involution occurs in the IPP.

### 8.3.2. Histology and lymphocyte subsets

At birth, JPP and the IPP have similar morphology. During its rapid growth, the IPP develops distinctive features [109]. The lymphoid follicles become ovoid and the interfollicular areas and domes smaller [25]. This coincides with larger numbers of B lymphocytes and very few T lymphocytes in the lymphoid follicles of the IPP. After the involution of the IPP has started, these differences disappear. Functional studies on lymphocyte traffic revealed major differences in the extent of immigrating lymphocytes: large numbers of lymphocytes immigrate into JPP, whereas little cell traffic is found in the IPP with the exception of the 10 to 20 cm next to the ileocecal junction where entry is as high as in JPP [87]. The protective effect of the orally applied lung-specific bacterium *Actinobacillus pleuropneumoniae* in a subsequent aerosol exposure of an LD<sub>50</sub> of this bacterium can be taken as an example of the integrated mucosal immune system and its relevance in future vaccine strategies in farm animals [44]. In a recently published study, four different subsets of dendritic cells were characterized in JPP, lamina propria, gut lymph and mesenteric lymph nodes of pigs, and the functional relevance is outlined for future vaccine studies [14].

In the colon, a patchy accumulation of lymphoid nodules is present at the ileocecal opening. ILF are present in all parts of the large intestine with an increased frequency in the central colonic flexure. The first ILF were identified at day 95 of gestation; at birth about 600 ILF and in 1 to 3 month old pigs more than 1000 ILF were counted [15, 50, 80]<sup>2</sup>. Lymphoid nodules in the colon have been termed LGC, since they often

consist of several lymphoid follicles and interfollicular areas in the submucosa with several radially branching crypts extending into the lymphoid tissue [80].

### 8.3.3. Morphology and function of the lymphoepithelium

LE occurs on JPP domes, IPP domes and in LGC in the large intestine [26, 73, 80, 122]. It consists mostly of enteroabsorptive cells and only a few interspersed M cells. In the LE of JPP more M cells are present than in the LE of the IPP. In the crypt epithelium of LGC, patches of LE with M cells can be found. M cells in the large intestine are more variable in appearance than in the small intestine. Uptake of ferritin and HRP was demonstrated for M cells on JPP and IPP domes [27, 73]. Uptake of ferritin by M cells in LGC was at a similar rate as by M cells in the small intestine [73].

## 8.4. Equine

GALT in horses has received considerably less attention than MALT structures of the upper and lower respiratory tract in this species.

### 8.4.1. Distribution and amount

Peyer's patches are present in the jejunum and ileum and ILF in the large intestine (Fig. 3D).

PP develop during gestation and in the newborn 245 to 320 JPP have been counted [19]. This number remains constant during the first years of age. In adult horses, 100 to 200 JPP of highly irregular shape were described [118]. In the ileum, an IPP is encountered that is 20 to 35 cm long in the newborn, increases in size in young horses and disappears in older horses [19]<sup>3</sup>.

<sup>3</sup> May H., Vergleichende anatomische Untersuchungen des Lymphfollikelapparates des Darmes der Haussäugetiere, Vet. Med. Thesis, Univ. Gießen, 1903.

In the large intestine, ILF are diffusely distributed from the cecum to the rectum (Fig. 3D). Their number is increased in the cecum where they may form a circular, 10 to 20 cm long accumulation in the cecal apex, in the pelvic flexure, in the left dorsal colon and along the anal ring [19]. In the cecum, on average about 25 000 nodules were calculated to be present in 2 to 6 year old horses<sup>4</sup>. The number regressed to about 14 000 nodules in horses over 16 years of age<sup>4</sup>.

#### 8.4.2. Histology

Lymphoid nodules in the IPP of horses exist in morphologically different forms as follicle-dome structures, propria nodules and LGC [75]. Carlens (1928) described the ILF in the large intestine as lymphatic crypts and propria nodules. Ripke<sup>4</sup>, in contrast, identified only follicle-dome units in the cecum with lymphoid follicles predominantly located in the submucosa and lymphocyte infiltrates extending dome-like to the apical level of the colonic mucosa.

#### 8.4.3. Morphology of the lymphoepithelium

In the IPP, LE with M cells has been described [75]. Lymphoid nodules in the cecum are covered by LE with M cells as identified by transmission electron microscopy<sup>4</sup>. No functional studies have been performed yet.

### 9. CONCLUSIONS

Knowledge about MALT in farm animals covers mostly the distribution; less is known about the morphology and even less about the function. Some sites, such as LDALT, DALT, SALT, where MALT is

present in laboratory animals or humans have not been investigated in farm animals yet. The information available might be used to select species as models for humans. Although research involving farm animals is cumbersome, they might be better comparable to the human situation as far as the distribution of MALT is concerned and because they are mainly kept under conventional conditions and not under the artificial SPF or germ free conditions as laboratory rodents. On the other hand, knowledge about MALT should be used to develop and improve oral, nasal and conjunctival vaccines in order to induce better protection of mucosal surfaces in the respective farm animals.

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<sup>4</sup> Ripke A., Zu Anzahl, Größen und Verteilung der Lymphknoten und der schleimhaut-eigenen Lymphknötchen am Caecum des Pferdes, *Vet. Med. Thesis, Veterinary School Hanover*, 1997.

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