Cellular Basis of Chronic Obstructive Pulmonary Disease in Horses

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Chronic obstructive pulmonary disease (COPD) is an inflammatory obstructive disease of the airways characterized with hypersensitivity of the airway tissues to various allergens, most commonly the fungi contained in the poor-quality hay and straw bedding—Saccharopolyspora rectivirgula, Aspergillus fumigatus, and Thermoactinomyces vulgaris. It is manifested clinically in middle-aged horses with recurrent episodes of dyspnea, chronic cough, and their reduced athletic and working capacity. Pulmonary emphysema and lack of pulmonary collapse are the most common gross lesion. Pathohistological findings in horses with COPD are chronic bronchitis/bronchiolitis, with characteristic changes in lumen, mucosa, submucosa, and smooth muscle layer and alveolar emphysema, both distensive and destructive form. Increased immunoreactivity in lungs and tracheobronchial lymph nodes is also noted. Most common lesions seen on cytology imprint smears from tracheal bifurcation is thick, viscous, PAS-positive mucus that forms Curschmann’s spirals. Dominant cell population consists of desquamated airway epithelial cells, as well as eosinophils, neutrophils, mast cells, erythrocytes, and alveolar macrophages. Primary pulmonary pathogens as well as potential contaminants and secondary infection agents were isolated bacteriologically from lung samples. All of the aforementioned findings correlate pointing to the fact that chronic bronchitis/bronchiolitis represents a basic substrate of COPD, which have combined inflammatory and immunological etiology, and emphysema is secondary to airway obstruction.

KEY WORDS: Horse, Lungs, COPD, Pathohistology, Cytology.
I. Introduction

Because respiratory diseases are widely spread among the horse population and account for a substantial share of pathology of the animal species, study of chronic obstructive pulmonary disease (COPD) in horses is of great importance. COPD belongs to the group of chronic respiratory diseases that reduce the value of the sports animals and working capacity of the draught animals. The importance of the study is further illustrated by the fact that COPD is markedly similar to allergic, atopic, extrinsic asthma in humans. Study of pathogenesis and therapy of the COPD in horses may contribute to the body of knowledge in pulmonology and promote treatment of asthma and chronic inflammatory disease of airways occurring in humans hypersensitive to various known and unknown environmental stimuli. COPD is an inflammatory obstructive disease of the airways characterized with hypersensitivity of the airway tissues to various allergens, most commonly the fungi contained in poor-quality hay and straw bedding (S. rectivirgula, A. fumigatus, and T. vulgaris). It is manifested clinically in middle-aged horses with recurrent episodes of dyspnea, chronic cough, and their reduced athletic and working capacity.

The disease is commonly called pursy or chronic alveolar emphysema, but there are many synonyms, because all authors studying this issue proposed a name for the disease: heaves, broken wind, RAO (Recurrent Airway Obstruction), chronic bronchiolitis-emphysema complex, chronic small airway disease, alveolar emphysema, chronic bronchiolitis, allergic bronchiolitis, asthma, asthmatic bronchiolitis, chronic cough, chronic pulmonary emphysema, chronic bronchitis/bronchiolitis, chronic pulmonary disease, hypersensitive pneumopathy, hyperreactive airway disease, chronic airway reactivity, and hay sickness (Rooney and Robertson, 1996).

This disease is encountered in horses that spend a lot of time in poorly aired dusty stables during winter, although there is a special type of the disease that also occurs in the summer. The reference literature states that the age at which the disease occurs for the first time varies from 4 to 8 years or more. It is most frequently encountered in sports horses in the following disciplines: show jumping, equestrian dressage, endurance (endurance race, cross-country riding with regular veterinary check-ups in which the horse pulse must not exceed 64 beats per minute), eventing (comprises show jumping, dressage, and cross-country with show jumping), but it also occurs in horses that are used for recreational riding, in riding schools, etc. (Art et al., 1998).

The disease presents itself in two forms: (1) typical form of COPD in the winter months in horses that spend longer time in unaired, dusty stables and
are hay-fed; and (2) Summer pasture-associated obstructive pulmonary disease (SPAOPD)—this form of the disease is encountered in the southeast part of the United States, California, and the United Kingdom during the summer when the horses are grazing and when the weather is warm and humid (Costa et al., 2001; Robinson, 2001; Seahorn and Beadle, 1993). Both in humans and horses the genetic base of COPD is considered to be very important. In the pathogenesis of equestrian COPD, an important role is played by T lymphocytes, CD4+ Th1 that release: IL-8, MIP-2, LTB-4, and ICAM-1, whereas CD4+ Th2 lymphocytes produce IL-4, IL-13, and IL-5.

Pathogenesis of the disease has not been fully elucidated, but some hypotheses have been proposed suggesting the development of the disease. With genetic predisposition, noxae (recurrent and uncured viral and bacterial infections of the airways, noxious effect of protease, and endotoxins) lead to lesions on the airway epithelium—loss of the cilia from the ciliary epithelium, desquamation of the epithelial cells of bronchioli and bronchi, as well as denudation of the basal membrane. Denudation of the basal membrane enables the antigen to establish a direct contact with the immunologically active tissues and as a result respiratory tissues become hypersensitive (McPherson and Lawson, 1974; Moore et al., 2004; Trailovic, 2000).

The main role of CD4+ Th1 that produce IL-4, IL-5 and IL-13 activated mastocytes, platelets, epithelial cells, and substances originating from these cells—histamine, bradykinin, LTC-4, LTD-4, PAF, PGD2, PGF2α—has been recognized in the pathogenesis of asthma. These substances lead to a series of pathological changes characteristic of both diseases. Macroscopically, appearance of the lung is mainly unchanged, but some authors suggest that in horses suffering from COPD the lungs usually do not collapse at exenteration from the chest or appear voluminous and excessively inflated, pale pink, and occasionally with imprints of the ribs seen. Sometimes there is emphysema as well as thick mucus that may be squeezed out of the lung section when pressed.

The pathohistological findings are very characteristic. The main pathohistological substrates of COPD include bronchitis and bronchiolitis that are characterized with changes on the mucosal and muscular layers of bronchi and bronchioli, in the peribronchial and peribronchiolar tissues as well as accumulation of content in the airways, their obstruction, and consequent development of secondary emphysema and atelectasis. Regardless of the fact that the disease was recognized long ago, few data exist on the pathogenesis, which makes the diagnosis establishing difficult. In addition to standard diagnostic methods, cytological smears obtained by bronchoalveolar and tracheobronchial lavage are also used. Moreover, imprints from the mucosal tissue in the tracheal bifurcation make postmortem diagnostic substantially easier and substantiates the diagnosis of the disease.
II. Morpho-Functional Features of Horse Lungs

A. Morphology

1. Lungs

Histology of the lungs is composed of conducting elements (lung conducting ways) composed of the bronchi and bronchioli; transitory elements composed of respiratory bronchioli; respiratory elements comprising alveolar channels, alveolar sacs, and alveoli; and stromal elements of the lungs represented by vascular and lymph vessels and nerves. The conducting elements start where the trachea branches into the right and main left bronchus that are initially extrapulmonary situated where in the lung hilar region they are covered by the lung parenchyma and continue to branch further intrapulmonarily. The conducting elements of the lungs are composed of the primary, secondary, and tertiary bronchi.

The wall of the extrapulmonary bronchus is similar to the tracheal architecture. The mucosa has an epithelial layer \( (\text{lamina epithelialis mucosae}) \) that is pseudostratified columnar, ciliated epithelium composed of stem, ciliary, goblet, and basal cells situated on the basal membrane \( \text{(Banks, 1993)} \). Below the basal membrane there is \( \text{lamina propria mucosae} \) —submucosal body, composed essentially from connective tissue rich with elastic fibers. The third layer is composed of elastic fibers, and it is believed to play the role of mucosal muscular layer. This is called lamina elastica mucosae. The \( \text{lamina propria} \) accommodates branched tubuloalveolar glands that spread in the cartilage and lymph follicles. The cartilage has a horseshoe shape. At the places where the cartilage ring is discontinued transversally spread smooth muscles may be seen. The whole extrapulmonary bronchus is covered with adventitia composed of connective tissue.

The intrapulmonary bronchus architecture differs somewhat from the extrapulmonary bronchus. The epithelial mucosal layer is also a pseudostratified in the type and mutual disposition of the cells present. It is also composed of stem, ciliary, goblet, and basal cells that cover the basal membrane. The fur is basically composed of elastic fibers. The smooth muscle fibers are distributed spirally and comprise the muscular layer of the mucosa \( (\text{lamina muscularis mucosae}) \). The submucosa is composed of connective tissue rich with collagen fibers accommodating the branched bronchial glands \( (\text{glandulae bronchales}) \) that are a combination of serous and mucinous cells, where the number of these cells decreases toward the tertiary bronchi. The submucosa also accommodates lymph nodes, that is, follicles \( (\text{lymphonodi bronchales}) \), nerve fibers, and ganglia of the neurovegetative system, blood and lymph vessels.
The bronchial cartilage in larger bronchi is semicircular but the cartilage rings get smaller and take the plaque shape or disappear completely at the site where the tertiary bronchi become the primary bronchioli. The epithelial mucosal layer of the bronchioli is composed of a single layer of squamous of columnar ciliary epithelium, where the number of cilia is higher in the primary, lower in the secondary bronchiole, whereas the tertiary bronchioli have no cilia at all. In addition to the ciliary, there are also cells without cilia called the Clara cells. The goblet cells are scarce. The bronchial and bronchiolar mucosa also contains neuroendocrine cells (in man called Feyrerter, or Kulchitsky-like or K-cells) that exhibit neurosecretory type granules and contain bombesin, calcitonin, and in fetal lungs somatostatin (Banks, 1993; Rodriguez et al., 1992). The lamina propria is composed of elastic and collagen fibers where mastocytes may be seen (Young and Heath, 2000). There is no cartilage in bronchioli.

Respiratory bronchioli in horses, as opposed to humans and some other species, are poorly developed and represent transitory elements of the lungs, establishing the link between the tertiary bronchioli and alveolar ducti and gas exchange takes place in them, as well. The epithelial layer of mucosa is columnar, the lamina propria is rich with elastic and collagen tissues with a layer of smooth muscles.

The respiratory elements of the lungs include alveolar channels (ductus alveolaris), alveolar sacs (sacculus alveolaris), and alveoli (alveolae pulmonales). The respiratory alveolae at the end split into numerous alveolar channels that are composed of alveoli. The alveolar channels also split and spread peripherally into the alveolar sacs. The alveolae are composed of two types of cells, pneumocytes type 1 (membranous pneumocytes, flat cells whose nucleus partially protrudes into the alveolar lumen) whose role is to communicate between air and blood to enable gas exchange, and pneumocytes type 2 (granular pneumocytes, circular or square cells that protrude into the alveolar lumen). Their role is secretory, meaning they produce and secrete pulmonary surfactant. The pulmonary surfactant is a secretory product of pneumocytes type 2 and is a kind of detergent primarily composed of a substance that reduces the surface tension called dipalmitoyl phosphatidyl choline (Young and Heath, 2000).

The lungs are permanently exposed to the influence of foreign materials inhaled with the air where the pulmonary macrophages (together with mucociliary apparatus of the lungs and protective substances in the bronchial fluid) are one of the defense mechanisms the body uses against foreign materials. Two types of pulmonary macrophages have been described, septal cells and alveolar macrophages where both conduct phagocytosis of foreign materials. The air-blood barrier is composed of pneumocytes, alveolar basal membrane, septal space, basal membrane of the blood vessels, and vascular endothelial vessels (capillaries).
The stromal elements of the lungs are composed of blood vessels, lymph vessels, and nerves (Banks, 1993). The immune system of the lungs is represented with six different types of lymphatic tissue: (1) free luminal lymphocytes present in smaller number in small bronchioli and alveolae; (2) intraepithelial lymphocytes present in the bronchi and bronchioli; (3) isolated lymphocytes in the mucosal fur that may be found in bronchi and bronchioli; (4) areas of densely packed lymphocytes occasionally present in small intrapulmonary bronchi; (5) lymphoid tissue with lymph noduli that may also be occasionally found in small intrapulmonary bronchi 4- to 8-mm wide (Mair et al., 1987). As opposed to the local bronchus-associated lymphoid tissue (BALT), the presence of bronchiole-associated lymphoid tissue (BRALT) has been evidenced (Mair et al., 1988); and (6) the last type of immunologically active tissue is represented with lymph nodes represented by the bronchial lymph center (lymphocentrum bronchale).

2. Lymph Nodes

In the course of embryonal development the lymph nodes develop after the thymus and spleen. The lymph nodes develop from the periarterial mesenchyme. Development of lymph vessels is followed by links between the lymph nodes and arteriolae where clusters of lymph cells occur, and periarteriolar reticular cells form a network that is an adequate environment colonized by lymphoblasts originating from the bone marrow or thymus. Foals are born with their lymph nodes formed, and in case of the presence of intrauterine infection they have germinative centers, as well (Valli, 1985).

The lymph nodes are clustered, encapsulated lymphatic tissues. The lymphatic tissue is encapsulated and from the capsule to the inner part of the lymph node, the partitions ( trabeculae) are spread. The capsule and trabeculae are composed of connective tissue rich with collagen fibers, whereas the stromal elements are composed of reticular fibers secreted by the reticular cells (probably fibroblasts). These fibers, together with the cells, form a thick network within the lymph node. Formation of this network is supported by dendritic cells that are characterized with numerous cytoplasmic protrusions. These cells play the role of antigen presenting cells (APC) together with macrophages and exceptionally B lymphocytes, and depending on the localization site, they are termed interdigititation cells in the T-cell area of the lymph node, or follicular dendritic cells (FDC) in the B-cell zone. The lymphatic system is organized as primary and secondary lymph nodes (follicles). The primary follicles are composed of densely packed small lymphocytes. The secondary follicles have a central light region composed of macrophages and large lymphocytes with light cytoplasm and light chromatin in the nucleus, and the area is called the germinative center. The germinative center is
surrounded by the darker zone (mantle zone, corona) composed of small lymphocytes with reduced cytoplasm and darkly stained nuclear chromatin.

The lymph node cortex is divided into the nodular area accommodating the lymph follicles, internodal zone, and deep zone. The internodal and deep zones make the paracortical zone or paracortex. In horses, fusion of the follicles (nodular fusion) frequently takes place.

B lymphocytes are situated in the primary follicles and germinative centers of the secondary follicles, and the T lymphocytes are situated in the paracortex. The lymph node center, that is, medulla composed of branched trabeculas, reticular fibers, and cells (lymphocytes, plasmocytes, and macrophages) surrounded by the medullar sinuses and lymph capillaries, where these formations are termed the medullar bands. The afferent fibers of the lymph nodes enter the lymph node capsule in the medullar sinus. Lymph passes through the cortical sinuses, follicles into the medullar sinuses that subsequently merge and compose efferent lymph nodes that leave the lymph node in the hilar region.

In the hilar region arteries branch through the lymph node trabeculas to the capillary level entering a lymph node and venous drainage proceeds. The postcapillary venules play a role in lymph recirculation from the blood. Lymphocytes leave the blood through these and enter the follicles (B lymphocytes) or paracortex (T lymphocytes). These cells leave the lymph node via the efferent lymph vessels and via the thoracic ductus enter the venous system and subsequently, when they pass through the heart and small pulmonary circulation, they enter the systemic circulation. From the systemic circulation they re-enter a lymph node, and the cycle is repeated (Banks, 1993; Heath and Perkins, 1989). Lymph nodes participate in production of lymphocytes, lymph filtration, phagocytosis of foreign matter, and production of antibodies.

B. Physiology

Respiratory organs may be divided into the upper airways, respiratory muscles, chest wall and lungs, that is, production elements (conducting pulmonary ways) composed of the bronchi and bronchioli; transitory pulmonary elements composed of the respiratory bronchioli; and respiratory elements of the lungs including alveolar channels, alveolar sac, and alveolae (Banks, 1993). The upper respiratory airways comprise the nasal cavity, paranasal cavities—sinuses, nasopharynx, and trachea. The upper respiratory ways that together with the conducting parts of the lungs (bronchi and bronchioli) represent the “anatomically dead space” (in horses weighing 450 kg it amounts to 1.5–2.0 liters of air) meaning space in which no respiration takes place, but heating of the inspired air to the body temperature, enrichment of the air with humidity and its purification from larger particles, exceeding the
size of 5 μm that are halted in the nose and expelled into the external environment by secretion, while particles sized <5 μm and >0.5 μm enter the lower conducting parts of the lungs and are also expelled into the external environment by expired air, cough, bronchial or bronchiolar secretion (mucociliary lift), or phagocytic activity of alveolar macrophages (Art et al., 2002). During inspiration, the inspired air is mixed with the air from the “physiologically dead space” composed of “anatomically dead space” (conducting air space) and “alveolar dead space” (space in the alveolae in which there is air but no gas exchange) so that the air from the atmosphere is not inspired alone, but mixed with the air from these spaces. Respiration is supported by the inspiratory muscles and expiratory muscles. The inspiratory muscles, diaphragm, mm. intercostales externi, mm. scalene, m. sternomandibularis help increase lateral, cranio-caudal and dorso-ventral diameters of the chest during inspiration and, consequently increase its volume, whereas the nasal muscles—m. levator nasolabialis, m. caninus, mm. nasales (m. dilatator naris apicalis and m. lateralis nasi) support preservation of the nasal diameter necessary for respiration because horses breathe through the nose.

Maintenance of the necessary diameters of the upper and lower airways is supported by the rigid structures such as the cartilage in the trachea and bronchi that prevent collapse of these structures. Interaction of the aforementioned muscles and pleura results in increased volume of the chest and negative intrapleural pressure, resulting in increased volume of the lungs, reduced intrapulmonary pressure, and when the value lower than the atmospheric one is reached, the air from the atmosphere via the conducting ways is “sucked into” the lungs. The suction is resisted by the elasticity of the lungs but the lungs spread, nevertheless, because the power of the inspiratory muscles exceeds that of the elasticity of the lungs, but during the inspirium, potential energy is deposited in the lungs and subsequently used for the expirium. Owing to that, the horses use the total of 2–5% of the total body energy for breathing, according to the state (rest or exercise). The expirium is mostly a passive process, but the expiratory muscles nevertheless take part: mm. intercostales interni as well as abdominal muscles that adhere to the ribs—m. obliquus externus, m. rectus abdominalis, m. transversus. The intrapleural space is the space between the parietal and visceral pleural leaves, where the intrapleural pressure is, which may be either negative, (i.e., lower than the atmospheric pressure during inspirium) or positive, (i.e., higher than the atmospheric one during expirium).

During amble and trot the frequency of trot and breathing are not related, although some studies still suggest that they may be coordinated, whereas in the gallop the functions are always related (Art et al., 2002). Normal respiratory frequency in horses is between 8 and 16 respirations per minute (may reach 110–130 during exertion, or maximum up to 148 respirations per minute). The largest amount of blood in horse lungs is situated in the dorsal parts of the
lungs, instead of the ventral ones as believed previously, because gravitation plays only a minor, almost negligible influence on distribution of blood in the lungs (Art et al., 2002). The lungs play a role in respiration and maintenance of acid-base balance, metabolic function, endocrine function, defense function, thermoregulatory function, and excretory function—expelling volatile substances from the circulation: alcohol and acetone bodies, volatile anesthetics, methane, and other gases.

Breathing is regulated by activity of respiratory centers that are situated in the area of medulla oblongata and pons. There are four of these: inspiratory, expiratory, apneustic, and pneumotaxic. These centers are controlled by the higher parts of the vegetative nervous system—hypothalamus and limbic cortex. The inspiratory center is presented with the dorsal group of neurons of the medulla oblongata, belongs to the neurons of tractus solitarii that represents nuclei of the VII, IX, and X cranial nerves. It is tone-active, and receives the information from numerous chemo, baro, stretch, and other receptors. It spontaneously produces 8–16 rising signals per second.

The ventral group of neurons of the medulla oblongata operates concomitantly as the inspiratory and expiratory center, because it innervates both inspiratory muscles (mm. intercostales internii), and expiratory muscles (mm. intercostales externii and mm. abdominales). The apneustic center is situated in the lower part of the pons, it is also tone-active and is expected to prevent interruption of the rising signal from the inspiratory center. The pneumotaxic center is situated in the pons and is required to inhibit the apneustic and, indirectly the inspiratory center. It interrupts the inspirium and regulates the frequency and rhythm of respirations.

III. Cytological Features of Equine Lungs

The pulmonary parenchyma contain the subepithelial and free mastocytes that have, on the surface, incorporated E class immunoglobulins (IgE) and as a response to stimulation by specific antigen release histamine, heparin, arachidonic acid metabolites, platelet activation factor, and hemotoxic factors. Consequently, they play an important role in pathogenesis of some major equine diseases such as COPD and infection by pulmonary nematodes (Ainsworth and Biller, 1998).

Secretion of the lower airways reveals various kinds of cells. The cytologic finding is one of the most important parameters for diagnosis of various respiratory diseases. In clinically healthy horses the usual cytological finding in secretion originating from lower airways comprise usually sparse lymphocytes, macrophages, few cells from the bronchial epithelium, and a small amount of mucus. In horses suffering from miscellaneous diseases of the
bronchi, bronchioli, and alveolae, one may find macrophages, lymphocytes, neutrophil granulocytes, mastocytes, eosinophil granulocytes, erythrocytes, desquamated cells of bronchial and bronchiolar epithelia. In addition to the cells, smaller or larger amounts of mucus may also be found, as well as the bacteria that may be intracellular or extracellular, or may even form colonies.

The cytological smear presents alveolar macrophages as large cells, 15- to 40-μm diameter with high cytoplasm: nucleus ratio, 3:1. These cells are frequently vacuolized and may contain phagocytosed cellular debris (phagocytosed erythrocytes, hemosiderin, desquamated epithelial cells, apoptotic cells, fungal spores, pollen grains, etc.). The alveolar macrophages of horses present a low level of expression of major histocompatibility complex, class II antigen (MHC-II), and accessory molecules CD80 and CD86, so that presentation of antigen CD4 T cells is poor (Horohov, 2004).

Lymphocytes are cells with large oval- or kidney-shaped nucleus. There are two populations of lymphocytes: small, about 6-μm diameter, and larger, whose diameter is between 10 and 15 μm. Both types are encountered in clinically healthy horses and those suffering from respiratory diseases. There are two types of lymphocytes: (1) B lymphocytes that after activation are differentiated into M cells, memory cells and plasmocytes that produce specific antibodies (immunoglobulins) by way of which the B lymphocytes participate in humoral immunity; (2) T lymphocytes that may be Tc (cytotoxic) that may by way of substances they excrete (granzymes, perforines, TNF-β) directly kill microorganisms, virus-infected cells, and tumor cells; and Th (helper) lymphocytes that after contact with a certain antigen presented by macrophage (antigen presenting cell [APC]) depending on the subpopulation to which they belong exert effect on Tc lymphocytes or B lymphocytes (Th1 lymphocytes via cytokine IL-2 and TNF-β to Tc lymphocyte by potentiating their effect, and Th2 via IL-4, IL-5, IL-6, and IL-10 to B lymphocytes stimulating them to differentiate into memory cells and plasmocytes that produce immunoglobulins) (Banks, 1993). T lymphocytes also produce interleukin IL-8, interleukin MIP-2 (macrophage inflammatory protein 2), leukotriene LTB-4, and integrin ICAM-1, interleukins IL-4, IL-13, and IL-5 (Beadle et al., 2002; Bowels et al., 2002; Cunningham, 2001; Francini et al., 2000; Geisel and Sandersleben, 1987; Giguere et al., 2002; Halliwell et al., 1993; Lavoie et al., 2001, 2002; Mair et al., 1988; Robinson, 2001; Schmallenbach et al., 1998). Most lymphocytes (90%) found in secretion of the lower airways of horses are T lymphocytes, whereas B lymphocytes account for only 10%.

Neutrophil granulocytes are white cells, sized 10–12 μm and contain segmented nucleus. A nucleus of a mature neutrophilic granulocyte usually has three lobes, although the number of segments may vary by the age of the cells from 1–2 segments in young cells (shift to the left) to 4–5 segments in older cells (shift to the right). Cytoplasm of these cells contain granula filled with matter endowed with bactericidal activity (lysozyme, lactoferrin, and
defensins), proteolytic action (cathepsin, collagenase, and elastase), lipolytic action (phospholipase A1 and phospholipase A2), etc. (Kaneko, 1998).

Mastocytes (mast cells) are oval cells that may vary in size with small and light, centrally situated nucleus. Their cytoplasm is filled with secretory granules that contain heparin, histamine, serotonin, leukotrienes, platelet activation factor, and eosinophil chemotactic factor (ECF). Eosinophilic granulocytes are cells sized 10–15 µm with bilobar nucleus and large specific granules that in horses may reach the size of 1–2 µm (diameter) and are called Seamers granules and are stained red with eosin. These granules contain the main base protein, peroxidase, hydrolytic enzymes, acid phosphatase, aryl sulphatase, and collagenase (Zinkl, 2002). Epithelial cells originate from epithelial layer of bronchial and bronchiolar mucosa and are mostly highly columnar ciliary or less commonly low columnar without cilia (originating from tertiary bronchioli) (Hewson and Viel, 2002).

### IV. Chronic Obstructive Pulmonary Disease (COPD) in Horses

In the respiratory system pathology COPD plays a very important role. COPD is an inflammatory obstructive disease of the airways that becomes clinically manifested in middle aged horses as recurrent episodes of dyspnea, chronic cough, and impaired sports and working capacity of horses (Robinson, 2001).

**A. Etiology and Pathogenesis**

**1. Etiology**

Etiology of the disease has not been fully elucidated but several factors are considered important for its occurrence.

Genetic predisposition plays an important role in the pathogenesis of the disease—studies conducted in two horse farms indicate a higher incidence of the occurrence of COPD in horses with one or both parents affected. It is suggested that numerous different genes participate in the occurrence of the disease. Genetic basis of the diseases has certain similarities with genetic basis of asthma in humans (Marti and Ohnesorge, 2002). Recurrent, inadequately manages bacterial and viral infections may also be considered in the etiology of this disease, particularly since correlation of the processes has been evidenced in both humans and experimental animals. Similar examples have been reported in horses, as well (Castleman et al., 1990; Lopez, 2001; McPherson and Lawson, 1974; Trailovic, 2000).
Toxins have also been proposed as a possible cause of COPD more specifically, endotoxins (Pirie et al., 2001) and exotoxins such as 3-methylindol (Derksen et al., 1982). In humans, occurrence of pulmonary emphysema is closely related to protease (i.e., deficit of antiprotease factors [congenital α1-antitrypsin deficiency] and smoking habit). In 1963, two Swedish researchers made a breakthrough in understanding the pathogenesis of lung emphysema in humans (Laurel and Ericsson, 1963). They have noted that serum α1-antitrypsin deficiency and increased incidence of lung emphysema coincide in families with this genetic defect. Emphysema that they noted was the very destructive panacinary emphysema without accompanying bronchitis that used to be called “idiopathic.” In horses also, activity of protease, both endogenous originating from neutrophilic granulocytes and epithelial cells and exogenous originating from microorganisms has been suggested, but it is believed they do not play as important of a role as in humans. Serine protease and matrix metalloproteinase 9 (MMP-9) have also been suggested (Gruning et al., 1986; Raulo et al., 2001; Winder et al., 1990). Pulmonary nematode Dictyocaulus arnfieldi has also been suggested as a factor that may contribute to development of this disease (Ainsworth and Biller, 1998).

Undoubtedly most important and most commonly suggested etiological factor is hypersensitivity of the affected horses to specific antigens, that is allergic reaction. Among the suggested allergens fungi are the most common; their spores in the air originating from poor-quality hay, dusty hay bed, and poorly aired, usually humid stables in which the fungi flourish. S. rectivirgula (until it was termed Faenia rectivirgula, and in older literature it was referred to as Micropolyspora faeni) plays the most important role in the occurrence of COPD (Derksen et al., 1988; Khan et al., 1985). In addition to this fungus, A. fumigatus and T. vulgaris are also frequently suggested (McGorum et al., 1993; Schmallenbach et al., 1998). Other allergens, considered potential causes of the disease are also suggested: βD-glucan (integral part of cell walls of fungi and bacteria), nonparasite mite (Lepidoglyphus destructor), pollen of plants, miscellaneous allergens from grazing fields, particles of plants and feed, allergy to chicken, etc. (Ainsworth and Biller, 1998; McGorum, 2001).

2. Pathogenesis

Pathogenesis of the disease has not yet been fully elucidated, but some hypotheses have been proposed suggesting the development course of this disease. In addition to genetic predisposition of noxae (recurrent or unhealed viral and bacterial infections of airways, noxious effect of protease and endotoxin) lead to lesions of airways epithelium—loss of cilia from the ciliary epithelium, desquamation of epithelial cells of bronchioli and bronchi, and
Denudation of the basal membrane enables direct contact of antigens with immunologically active tissues and consequent hypersensitivity of tissues in the airways (McPherson and Lawson, 1974; Moore et al., 2004; Trailovic, 2000). Hypersensitivity of airways is characterized by persistent bronchospasm after the contact between the bronchioli and bronchi with allergens. This process may last for several days after a single contact with allergens (Derksen and Robinson, 2002; Hoffman, 2001). In horses with hypersensitivity of airway mucosa presence of larger number of T lymphocytes is also noted (CD4+, CD3+), as well as eosinophilic granulocytes and mastocytes (Slocombe, 2001; Watson et al., 1997). When contact with the allergen takes place in these horses, combined allergic and inflammatory reactions occur.

Pulmonary alveolar macrophages (PAM) and T lymphocytes (CD4+ Th1) release cytokines: interleukin IL-8, interleukin MIP-2 (macrophage inflammatory protein 2), leukotriene LTB-4 and integrin ICAM-1, while CD4+ Th2 lymphocytes produce interleukins IL-4, IL-13, and IL-5. Interleukins IL-8, MIP-2, leukotriene LTB-4, and integrin ICAM-1 play a hemotoxic role so that neutrophilic granulocytes accumulate in the lumens of bronchioli and bronchus (Cunningham, 2001; Francini et al., 2000; Lavoie et al., 2002; Robinson, 2001). On the other hand, interleukins IL-4 and IL-13 play an important role in the switching of B lymphocytes to production of IgE, whereas IL-5 is responsible for tissue migration of eosinophilic granulocytes. In horses in which increased hypersensitivity is noted, increased concentration of IgE, IgA, and IgG in the airways is also present. Although increased production of IL-5 by CD4+ Th2 lymphocytes is also noted, massive infiltration of eosinophilic granulocytes does not take place, however the predominant cellular population is that of neutrophilic granulocytes (Beadle et al., 2002; Bowels et al., 2002; Geisel and Sandersleben, 1987; Giguere et al., 2002; Halliwell et al., 1993; Lavoie et al., 2001; Mair et al., 1988; Schmallenbach et al., 1998). Also, nuclear transcription factor κB (NFκB) occurs, stimulating cytokine production, and thus accumulation of neutrophilic granulocytes. In addition to prolonged stimulation for accumulation, these neutrophilic granulocytes have prolonged apoptosis, they live longer, and this at least partially, explains the fact that after a single contact with allergen, development of allergic inflammatory process that last for several days ensues (Bureau et al., 2000). Some reports suggest that oxidative stress, that is, substances that are released during the oxidative stress (isoprostanes—arachidonic acid derivatives) may play a certain role in pathogenesis of this disease (Kirschvink et al., 2001). In affected horses increased level of nitrogen oxide synthetase (iNOS) has been recorded, playing a multiple role in inflammation (Costa et al., 2001). Manifestation of increased sensitivity includes release of a large amount of mucus from goblet cells and from subepithelial cells into the lumen of airways where it is mixed with accumulated neutrophilic granulocytes and
cellular debris composed of desquamated epithelial cells of the airways. In the course of the allergic-inflammatory process that is the basis for development of COPD in parallel two processes take place in connection with goblet cells and subepithelial glands. At the sites where goblet cells and subepithelial cells physiologically are not present or are present in small amount their multiplication occurs. The process is called goblet cell metaplasia. At the sites where they normally occur, their number is significantly increased representing hyperplasia of the structures. It is believed that increased secretion of mucus results from increased number of mucosal cells where the actual mucus production is normal, increased production of mucus or reduced mucociliary clearance because of changes in the ciliary apparatus or changes in the physical features of the mucus (namely mucus secreted in this disease is thick, viscous, and sticky) (Hotchkiss, 2001).

Via IgE, the level of which is increased in horses suffering from COPD, allergens adhere to mastocyte membrane, the number of which is also increased in these horses, resulting in their degranulation. Mastocyte degranulation from their cytoplasmic granules results in release of biogenic amines—inflammation mediators: histamine, arachidonic acid metabolites (prostaglandins and leukotrienes), platelet activation factor (PAF), serotonin, and hemotoxic factor (Hare et al., 1999). Hemotoxic factors stimulate accumulation of neutrophilic granulocytes in the airway lumen. These reactions support the suggestion that hypersensitivity reaction type 1 plays a role in the pathogenesis of COPD, although hypersensitivity reaction type 3 has also been suggested as one of the causes of neutrophilic infiltration (Halliwell et al., 1979; Lavoie, 2001; Lorch et al., 2001). Serotonin, histamine, and leukotriene D4 (LTD-4) increase sensitivity of smooth muscles to endogenous acetyl choline (Ach) released from activated parasympathetic nerves and bound to M3-muscarinic receptors on smooth muscle cells of the muscular layer of bronchi and bronchioli. Additionally, histamine and serotonin promote increased release of acetyl choline from nerves. Lesions on epithelium of bronchus and bronchioli result in reduced production of epithelium-derived relaxing factor (EpDRF) whose physiological function is to control reactivity of bronchioli and bronchi and reduce the capacity for bronchospasm. Combination of these factors results in bronchospasm—contraction of smooth muscles of bronchioli and bronchi. Repeated episodes of effect of the allergen and persistent bronchospasm eventually result in hypertrophy—thickening of the muscular layer of the airways, particularly that of bronchioli (Derksen and Robinson, 2002; Robinson, 2001; Venugopal et al., 2001; Wang et al., 1995).

Due to permanent irritation, proliferation of bronchial and bronchiolar epithelium ensues, and subsequently squamous epithelial metaplasia follows in which the sensitive columnar ciliary epithelium is replaced by squamous epithelium. The squamous epithelium is more resistant to noxae, but because
it is devoid of cilia, function of mucociliary apparatus is affected, hindering expectoration of mucus, neutrophilic granulocytes and cellular debris from the lumen of small into the large airways and out in the environment. Increased accumulation of mucus, accumulation of neutrophilic granulocytes, desquamation of epithelial cells, proliferation of bronchiolar and bronchial epithelium and its squamous metaplasia, thickening of the smooth muscle layer, edema of the airway wall in the acute stage, as well as disruption of the function of mucociliary apparatus result in obstruction of the airways, hindering airflow through them, particularly in the expirium. Consequently, increased accumulation of air in the alveolae results, which secondarily leads to development of initially distensive emphysema (previously called compensatory), and subsequently when alveolar wall structure and interalveolar septae are disrupted, leads to development of destructive emphysema, compromising substantially gas exchange. Thus, emphysema is secondary in nature, and results from obstruction of airways (Geisel and von Sandersleben, 1987; Lopez, 2001; McPherson and Lawson, 1974) (Fig. 1).

B. Morphological Features of COPD

1. Macroscopic Findings

Voluminous and expanded lungs, pale pink, were found in 27.45% of studied horses, from our study, which is in concert with reference literature (Marinkovic, 2005; McPherson and Thompson, 1983; Robinson, 2001; Rooney, 1970; Slocombe, 2001). The rib imprints were not found in the

![Diagram](https://via.placeholder.com/150)

**FIG. 1**  Diagrammatic presentation of the overlap between the chronic inflammatory condition and COPD in horses.
studied horses. Emphysema was found in 11.76% of examined horses, coinciding with reports of other authors (Gerber, 1973; Lopez, 2001; McPherson and Lawson, 1974; Schoon and Deegen, 1983; Tyler et al., 1971). Reference literature suggests that volume of the chest in deceased asthmatic patients is increased, the lungs are “inflated,” frequently with marks of ribs on the surface.

2. Pathohistology

In the material examined for this study, bronchitis/bronchiolitis of various degree was diagnosed in 100% of studied horses (Marinkovic, 2005, 2007), coinciding with reports of Bracher and associates (1991) (Figs. 2 and 3). Reference papers by different authors differ only slightly in the percentage of histologically verified bronchitis/bronchiolitis from 37.4% (Winder and von Fellenberg, 1987) and 38% (McPherson et al., 1978). Conversely, in a study conducted in Switzerland chronic bronchitis/bronchiolitis of various degrees of severity it was established in 62.3–100% of studied horses (Bracher et al., 1991). In all studied horses loss of cilia, degeneration, necrosis, and desquamation of epithelial cells were found to various degrees, again in concert with reference literature (Kaup et al., 1990a,b). Also, proliferation of bronchia/bronchiolar epithelium was recorded where these cells form papillomatous proliferations that protrude into the lumen as reported by many authors (Kaup et al., 1990a,b; McPherson and Thompson, 1983; Slocombe, 2001; Winder and von Fellenberg, 1987, 1988). In humans during respiration or coughing these proliferates may fall off and enter the cytological material (sputum, bronchioaspirate) in the form of clusters (i.e., Creola bodies) (Naylor, 1962; Naylor and Railey, 1964) (Fig. 4).

![Fig. 2 Chronic bronchiolitis with epithelial proliferation, desquamation, and necrotic epithelial cells in the lumen (HE, ×400).](image-url)
Because the cells in these clusters are degenerated, frequently vacuolated cytoplasm and karyorrhectic nuclei, they may suggest adenocarcinoma of the lungs (Farber et al., 1957). Careful observation reveals that some have residual cilia which adenocarcinoma cells never have. Although this finding is highly suggestive of asthma, it has been described in completely different
circumstances (e.g., sputa of workers in steel plants or sputa of pig breeders) (Djuricic et al., 2001; Plamenac et al., 1974). Due to chronic irritation squamous metaplasia (SM) developed in 7.84% of studied horses, in concert with reports of other authors (Robinson, 2001; Schoon and Deegen, 1983; Slocombe, 2003; Winder and von Fellenberg, 1988). Generally, findings of SM and other atypical proliferations of bronchial mucosa in persons exposed to various noxae (air pollution, smokers, miners in asbestos mines, truck drivers, etc.) are very common (Auerbach et al., 1961; Berkheiser, 1959, 1963a,b, 1969; Couland and Kourilsky, 1953; Farber et al., 1954; Lamb and Reid, 1968; Plamenac and Nikulin, 1969; Plamenac et al., 1972a,b, 1973, 1978, 1980, 1981; Saccomano et al., 1963, 1970; Sanderud, 1956; Weller, 1953). For a while, it was believed that there was a mutual link between degeneration and destruction of bronchial epithelium on the one hand and its SM and occurrence of lung cancer in humans on the other (Figs. 5–7).

Not every metaplasia is a precancer process and it may affect the respiratory epithelium widely (Auerbach et al., 1961; Knudtson, 1960; Nasiell, 1967; Sanderud, 1958) and may even be taken as a physiological process in geriatric population (Plamenac et al., 1970). It appears that carcinoma may develop in the bronchial epithelium regardless of the presence or absence of SM that may be considered as nonspecific reaction to various lesions that may or may not accompany cancerogenesis (Melamed et al., 1977). Undoubtedly, metaplasia and precancer states of the bronchial epithelium play a minor role in pathology of horses compared to humans (Zinkl, 2002).

Finally, sporadic cases of lung cancer in horses are reported as casuistic rarities (Schultze et al., 1988; Van Rensburg et al., 1989) whereas SM is not such a rare phenomenon. Hyperplasia of goblet, mucus-producing cells established in 64.70% studied horses was reported by a series of authors.

FIG. 5  Proliferation of bronchial epithelia with cellular atypia (HE, ×400).
Costa et al., 2001; Kaup et al., 1990a,b; McPherson and Thompson, 1983; Schoon and Deegen, 1983; Slocombe, 2003; Winder and von Fellenberg, 1987, 1988). In the lumen of bronchi and bronchioli of all studied horses accumulation of a large amount of thick viscous mucus that occasionally forms mucosal plugs obstructing the lumen of these airways, was reported by many other authors, as well (Costa et al., 2001; Kaup et al., 1990a,b; McPherson and Thompson, 1983; Robinson, 2001; Schoon and Deegen, 1983; Slocombe, 2003; Winder and von Fellenberg, 1987, 1988; Zinkl, 2002). In addition to hyperplasia of goblet cells, the increased amount of mucus in the lumen of bronchus is promoted by hyperplasia of subepithelial cells of the bronchi, as evidenced in 45.1% of these horses. In 60.78% of horses subepithelial structures revealed aggregation of lymphocytes, mastocytes, eosinophilic granulocytes, plasma cells, and macrophages that occasionally

![FIG. 6](image6.png)

**FIG. 6** Squamous metaplasia of the respiratory epithelium (HE, $\times$200).

![FIG. 7](image7.png)

**FIG. 7** Tracheal imprint: Cluster of squamous metaplastic cells (HE, $\times$1000).
form lymph follicles (Kaup et al., 1990a,b; Schoon and Deegen, 1983; Slocombe, 2001). Hyperplasia of goblet cells, subepithelial mucus-producing glands has been described in patients suffering from asthma with consequent production of a large amount of thick, viscous, sticky, PAS-positive mucus that makes Curschmann’s spirals (Jeffery, 2001). Eosinophilic granulocytes are a characteristic finding in the sputa of asthmatic patients, together with findings of Curschmann’s spirals and Sharcot-Layden’s crystal (resulting from degradation of eosinophilic granulocytes under the influence of their phospholipases). The crystals are usually not recovered from the conventionally processed histological and cytological preparations, but only from plain sputum smears. There is no purpose in special search of these crystals because it has already been established that they are regularly recorded in all pathological states accompanied with eosinophilia, including some tumors. The mentioned triad (eosinophilic granulocytes, Curschmann’s spirals, and Charcot-Layden’s crystals) have remained a characteristic laboratory finding in cases of asthma. It is also well known that cytological diagnosis of this disease is more complex and that Creola bodies is the most important one. Also, findings of the Curschmann’s spirals are not pathognomonic for asthma only: they may be found in numerous, other pathological conditions. Also, eosinophils and Charcot-Layden’s crystals have been noted in the sputa of patients with pneumonia, echinococcus, lung tuberculosis as well as in patients with lung carcinoma.

Subsequent studies have shown that mucosal spirals, eosinophils, and crystals are not characteristic of asthma only, but may be seen also in other pathological conditions, including the sputa of smokers, former smokers, and persons exposed to noxious inhalants (Djuricic and Plamenac, 1998; Djuricic et al., 2001; Plamenac et al., 1972a,b, 1974, 1979b, 1981, 1985; Walker and Fullmer, 1970). Studies of numerous authors (Beadle et al., 2002; Bowels et al., 2002; Geisel and Sandersleben, 1987; Giguere et al., 2002; Halliwell et al., 1993; Lavoie et al., 2001; Mair et al., 1988; Schmallenbach et al., 1998; Zinkl, 2002) have shown the predominant cellular population in the lumen of airways in horses is represented by neutrophilic granulocytes and desquamated epithelial cells, macrophages, and eosinophilic granulocytes may be seen. However, in the studied material of our research, desquamated epithelial cells and eosinophilic granulocytes with a large number of neutrophilic granulocytes were the predominant cellular population in the lumens of bronchi and bronchioli. As opposed to COPD in horses, in asthma the airways lumen has the predominant cellular population of eosinophilic granulocytes that are situated peribronchially. In addition to these cells, there are also lymphocytes, macrophages, mastocytes, and neutrophilic granulocytes. Thickening, hyalinization of the basal membrane of airway epithelium common in asthmatic patients has not been recorded in horses suffering from COPD (Huang et al., 1999; Tiddens et al., 2000), or have been noted in the material investigated within this study.
Hyalinization of basal membrane, epithelial changes, eosinophilic infiltration of the wall, hyperplasia of the glands and muscular wall of the bronchi are absolutely pathognomonic and allow decisive pathohistological diagnosis of bronchial asthma in human even without clinical substantiation in cases of sudden death without witnesses which may happen in cases of *status asthmaticus*. Hypertrophy of the smooth muscle layer is almost regular in airways of asthmatic patients (Huang *et al.*, 1999; Jeffery, 2001; Martin, 2001; Tiddens *et al.*, 2000).

Decisive cytopathological diagnosis of asthma is not possible, but may only be fairly reliably suggested. Hypertrophy of the muscular layer of the bronchi and bronchioli noted in 25.49% of studied horses has been reported by other authors, as well (Costa *et al.*, 2001; Robinson, 2001; Schoon and Deegen, 1983; Slocombe, 2001, 2003; Winder and von Fellenberg, 1987, 1988). Peribronchiolitis and peribronchitis diagnosed in 27.45% of studied horses as a characteristic and common finding in COPD has been suggested by other authors, as well (Costa *et al.*, 2001; McPherson and Thompson, 1983; Robinson, 2001; Watson *et al.*, 1997; Winder and Fellenberg, 1987, 1988). Intensive infiltration of the lungs by eosinophils has been recorded in 34 (66.66%) studied horses and reference literature links it with parasitic infections or within systemic eosinophilia (Dixon *et al.*, 1992; La Perle *et al.*, 1998; Latimer *et al.*, 1996; Nicholls *et al.*, 1978; Rooney and Robertson, 1996; Srihakim and Swerczek, 1978). Increased numbers of eosinophilic granulocytes, particularly in lung interstitium has also been suggested by other authors (McPherson and Thompson, 1983). Alveolar emphysema recorded in 70.59% of horses from our study were more common as distensive one (overinflation in 54.9%), and less commonly as destructive emphysema in 15.69% studied horses (Marinkovic, 2005, 2007), also in concert with other reports (Gerber, 1973; McPherson and Lawson, 1974; Schoon and Deegen, 1983; Slocombe, 2003; Tyler *et al.*, 1971).

American authors define human emphysema as a condition of the lungs that is characterized by abnormal and permanent increase of airways distally from terminal bronchioli, accompanied by destruction of their walls (American Thoracic Society, 1976; Thurlbeck, 1970). Expansion of airways not accompanied with destruction of the walls is, however, called overinflation, as is distension of airways after unilateral pneumectomy, which should more pertinently be called compensatory overfill of the airways, instead of emphysema (*emphysema compensatorium*). Some British experts (Fletcher, 1959; Reid, 1967) insist there are two categories of conditions of the lungs: dilation of the airways and dilation with destruction (distensive and destructive emphysema). As a consequence of airway obstruction accumulation of air in the lungs ensues, and secondary development of distensive emphysema follows, which may eventually evolve into destructive emphysema (Geisel and Sandersleben, 1987; Lopez, 2001; McPherson and Lawson, 1974). After emphysema has been
recognized in families with α1-antitrypsin deficiency, research has focused the possible role of tissue proteolysis as a mechanism in the occurrence of emphysema in humans (Laurel and Ericsson, 1963). In 1965, Gross and colleagues induced the occurrence of emphysema in the rat by intratracheal administration of proteolytic enzyme papain. In subsequent papers it has been shown that enzymes with elastolytic activity are particularly effective (Blackwood et al., 1973; Janoff et al., 1977; Snider et al., 1974). In an experiment, destructive emphysema was discovered in guinea pigs exposed to venom of spider Latrodectus tredecimguttatus (black widow), in absence of inflammatory changes on the parenchyma of the lungs and bronchi. Emphysema most probably resulted from hypoxia and hyperinflation (Ducic and Plamenac, 1984).

3. Immunohistochemistry

Numerous authors dealing with the problem of chronic respiratory infections within either immunodeficient and allergic diseases of humans and animals, or circumstances of mixed etiological attributes, have recognized in their work the distribution of lymphocyte subsets in the local lymphatic pulmonary tissue, airway epithelium, and mediastinal lymph nodes. In a study conducted by Watson et al. (1997) in all COPD-positive horses, a large number of CD3+ cells were identified in the airway epithelium, but replicate sections stained with CD4 and CD8 showed a few positively stained cells in the same region. This finding supports the presence of a population of CD4- CD8- CD3+ T lymphocytes in the pulmonary interstitial compartment of the horse (Watson et al., 1997). In their study, the authors used Mabs specific for equine cell surface antigens to label lymphocyte subpopulations in the tissues. Our experience suggests it is possible to monitor distribution of T and B lymphocytes even using the murine Mabs specific for human cell surface antigens (CD3, CD79), in the lungs and mediastinal nodes of horses and other types of mammals: dogs, cats, pigs, as well as poultry (Aleksic-Kovacevic and Jelesijevic, 2001; Aleksic-Kovacevic et al., 1999; Kovacevic, 1991; Velhner et al., 2001).

In an comparative study of normal, allergic, and nonallergic asthmatic individuals, nonallergic asthmatics had a significantly higher CD4:CD8 ratio and a significantly lower number of CD8+ T cells in their peripheral blood than did either the normal or the allergic asthmatic individuals (Walker et al., 1992). The ratio CD4:CD8 with prevalence of CD8 lymphocytes was also noticed in the local bronchial lymphatic tissue and mediastinal lymph nodes of immunocompromised cats with retroviral infections (Kovacevic, 1993; Kovacevic et al., 1997).

Our investigations of COPD-positive horses showed large amount of lymphocytes that express CD3- and CD79-positive reaction in subepithelial regions of bronchi and bronchioli. These two populations of lymphocytes were also present in peribronchial and peribronchiolar tissue and less in the
pulmonary interstitium, alveolar septi, and perivascular. CD79-positive lymphocytes were present mostly in the germinative center of the cortex of tracheobronchial lymph node and CD3-positive lymphocytes were present in the marginal region of follicle and paracortex of the lymph node. Positive immunohistochemical reaction was visible in the form of marginal red membrane precipitate both on CD3 and CD79 lymphocyte populations in the lungs and in the tracheobronchial lymph node (Marinkovic, 2005).

In subepithelial structures of bronchioli and bronchi, as well as in peribronchial and peribronchial tissues and to a lesser extent in the interstitium of the lungs, alveolar septa, and perivascularly a large number of lymphocytes with positive CD3 reaction were recorded, complying with the reports of Winder and von Fellenberg (1988). CD79-positive lymphocytes were noticed mostly in the cortical region of lymph node, germinative centers of hyperplastic follicles, whereas CD3-positive lymphocytes were noticed in the marginal follicular region and lymph node paracortex, as reported by Searcy (2001) and Valli (1985). Presence of CD3+ lymphocytes in samples with morphological and histological signs of COPD should be observed from the point of view of various interleukins important for the development of COPD in horses and asthma in humans.

4. Cytology

In imprint preparations from the tracheal bifurcation in the studied material, the predominant cellular population comprises desquamated columnar cells recorded in 98.04% of studied horses (Marinkovic, 2005, 2007), in concert with other reference reports (Beech, 1975; Hewson and Viel, 2002; Zinkl, 2002). Other authors report preserved, unaffected neutrophilic granulocytes as a predominant cellular population in COPD in horses (Beech, 1975; Couetil et al., 2001; Derksen et al., 1988; Hare et al., 1999; Hewson and Viel, 2002; Lavoie et al., 2001; Lorch et al., 2001; Robinson, 2001; Seahorn and Beadle, 1993; Zinkl, 2002). In the studied imprint preparation from the tracheal bifurcation these cells were recorded in 19.6% of the studied horses. Desquamation and lesions of the columnar epithelium with loss of cilia from these cells, ciliocytophthoria (CCP), was registered by Hewson and Viel (2002), as well (Fig. 8).

Irritating forms or abnormal columnar cells of the respiratory epithelium may be found in miscellaneous acute or chronic inflammatory processes on the lungs (i.e., bronchi). The cells lose their regular cylindrical appearance, become stout, with increased amount of cytoplasm, and occasionally hyperchromatic or picnotic nuclei. These cells illustrate a nonspecific response to irritation of any kind and the incidence of this phenomenon is most common in lung cancer patients (Koss, 1979). However, this lesion has also been described in singers, players of wind instruments, and people in advanced
age (Plamenac and Nikulin, 1969; Plamenac et al., 1970). CCP is a term introduced in 1956 by Papanicolaou to signify severe lesion of columnar epithelial cell with its destruction and separation of the cytoplasm to the part that contains the nucleus and the one with remaining cilia. The process is frequently associated with eosinophilia of cytoplasm or nuclei or occurrence of small inclusions. At the beginning, viral inclusions were suggested, because the occurrence of CCP was registered in people suffering from viral pneumonia (Papanicolaou, 1956). Later, the same occurrence in pathognomonic incidence was seen in patients with lung carcinoma and other pulmonary diseases, whereby it lost its specific significance (Koss, 1979).

It was also seen in children smokers (Plamenac et al., 1979), people exposed to air pollution (Plamenac et al., 1979), and even in neonates with hyaline membrane disease (Doshi et al., 1982). Eosinophilic granulocytes in cytological imprint preparations were recorded in a substantially larger number than in smears of healthy horses. Hewson and Viel (2002) suggest finding of these cells in cytological preparations is characteristic of COPD in horses. Conversely, Beech (1975) contests this and interprets findings of these cells in the smears by the presence of parasitic infection of the lungs. Erythrocytes recorded in 47.06% of the horses most probably result from inflammatory processes on the lungs or occurred as a result of blood aspiration when the animals were sacrificed (slaughtered). In 21.57% of studied horses alveolar macrophages were diagnosed, frequently with phagocytes bacteria, epithelial cells of phagocyted coal dust. The authors suggest that they occur in COPD horses less frequently than usual (Beech, 1975; Couetil et al., 2001; Derksen et al., 1988; Hare et al., 1999), although they account for the predominant cellular population in healthy animals. Findings of a larger number of mastocytes has been recorded in cases of immune hypersensitivity,
which is one of important features of COPD, in concert with reports of some other authors (Hewson and Viel, 2002), although some other authors suggest that COPD-affected horses have a reduced number of these cells (Couetil et al., 2001; Derksen et al., 1988; Hare et al., 1999). Presence of mucus was recorded in 94.12% of studied horses (Marinkovic, 2005, 2007), less commonly as disorganized mucus, and more commonly as Curschmann’s spirals composed of thick, viscous fluids producing spiral forms, networks, or bizarre shapes as described is some other published papers (Beech, 1975; Hewson and Viel, 2002; Zinkl, 2002).

The following bacteria were isolated from samples of lungs of horses with purulent pneumonia: *Streptococcus equi*, *Streptococcus pyogenes*, *Staphylococcus aureus*, as reported by some other authors (Chanter, 2002; Giguere, 2000; Harrington et al., 2002; Karlstrom et al., 2004; Leguillette et al., 2002; Rooney and Robertson, 1996). Lungs of the studied horses yielded *Enterococcus sp.* in 11.76%, *Enterobacter sp.* in 33.33%, *Citrobacter sp.* in 5.88%, *Klebsiella sp.* in 9.8%, *Proteus mirabilis* in 37.25%, *Pseudomonas sp.* in 54.9%, and *Escherichia coli* in as many as 92.16% of studied horses. Although these bacteria have been suggested as possible causes of pneumonia (Chanter, 2002; Leguillette et al., 2002) particularly in slaughtered animals or those subjected to postmortem examinations, their importance in secondary infections or contamination should not be overlooked (Ainsworth and Biller, 1998; Rooney and Robertson, 1996; Sweeney, 2002; Sweeney et al., 1991). *Candida albicans* was isolated from 5.88% of the studied horses (lung samples) and it plays a role in the etiology of pneumonia in immunocompromised subjects, frequently within a systemic infection (Ainsworth and Biller, 1998; Hutchison, 1994; Reilly and Palmer, 1994). Fungi *S. rectivirgula*, *A. fumigatus*, and *T. vulgaris* have been suggested in reference literature as the most important factors in the occurrence of COPD. In the studied material here, however, these fungi have not been isolated because these fungi are not lung pathogens. Instead, they grow on feed (poor hay and grain), straw bedding (hay and straw) for horses, and participate in etiology of the disease as allergens (Derksen et al., 1988; Khan et al., 1985; McGorum et al., 1993; Schmallenbach et al., 1998).

Finally, based on results of our study and reference literature we may suggest that pathogenesis of COPD in horses and lung emphysema is somewhat different from the same disease and asthma in humans in spite of numerous similarities. Namely, in humans the main role in etiology is played by chronic bronchitis (etiology in horses is different), in which the predominant provoking factor is the smoking habit, but one may not overlook adverse environmental influences and air pollution (the same applies to horses). Also, in humans, a very important role is played by α1-antitrypsin deficiency (emphysema without bronchitis), which is not the case in horses. There is no asthma in horses (at least not pathomorphologically) whereas in humans it represents an important component of COPD that in its pure form
(nonsmoker’s asthma) rarely results in emphysema. The influence of smoking (cigarette smoking primarily) in the development of emphysema in humans may be compared with the influence of poor-quality hay, straw bedding, and poor ventilation of stables in horses. In horses, the disease most probably begins with recurrent bronchitis that spreads, resulting in the development of more or less diffuse bronchiolitis, after which distension follows and subsequent destruction of alveolar spaces, that is, COPD is accompanied with emphysema with all pertinent consequences.

V. Conclusions and Perspectives

Cytological analysis of mucosal imprints of the tracheal bifurcation suggests the presence of an asthmatic pattern analogous to that of humans (a large number of eosinophils, mucosal spirals, Creola bodies) but histological examinations of pulmonary parenchyma and bronchi do not correspond to human asthma, because hyalinization of the basal membrane is missing (Dunnill, 1960; Salvato, 1968; Sanerkin and Evans, 1965). Obviously, allergic component plays a very important role in the pathogenesis of COPD. The diagnosis is established according to history, clinical presentation, general clinical examination, and specialized diagnostic procedures.

The history suggests this disease occurred usually in advanced age, 4- to 8-years old (sometimes even later). The owner or caretaker of animals usually recognize chronic cough as the most important complaint, usually intensified after exposure of the animal to the allergen (dust during feeding or cleaning in classic forms of COPD, or allergens in grazing fields in the form of SPAOPD). Horses breathe with difficulty, tire easily, and after the animals are exposed to physical activities, heart and respiration frequencies return to the physiological values slowly—the resting time is prolonged.

Clinical presentation is characterized with chronic cough, dilated nostrils, mucopurulent nasal excretion, accelerated respirations—tachypnea, prolonged insirium, abdominal breathing, “anal breathing,” abnormal respiratory sounds in the lungs (barely auscultable because of poor air flow, or wheezing), enlarged percussion area of the lungs, hypertrophy of abdominal muscles (m. obliquus externus abdominis)—“heave line,” and loss of body weight (even cachexia) resulting from the fact that they eat less.

The procedures used in the diagnostic of COPD include bronchoscopy, determination of blood gas levels (O₂, CO₂), cytology (BAL, TBL), radiography, intradermal allergic tests, allergic testing by nebulization of hay dust suspension (HDS), whole blood analysis, blood biochemistry, lung biopsy, etc. (Ainsworth and Biller, 1998; Hewson and Viel, 2002; McPherson et al., 1978; Pirie et al., 2002a,b,c; Robinson, 2001; Rose and Hodgson, 1993;
Therapy of the disease usually comprises systemic and inhalatory corticosteroids (e.g., dexamethasone, prednisolone) and bronchodilators (usually clenbuterol and albuterol, but some others as well). Bronchosecretolytic agents may also be used (dembrexin-chloride, acetyl cysteine), nebulization of physiological saline that is associated with bronchosecretolytic effect, hyperfusion, disodium chromoglycate (Chromelin), furosemide, antihistaminics etc. (Ainsworth and Biller, 1998; Robinson, 2001; Rose and Hodgson, 1993; Rush, 2001; Trailovic, 2000).

The main point in prevention of the disease and reduction of the associated clinical symptoms comprises reduction or complete elimination of exposure of the animals to the allergen that provokes the disease. This implies taking the animals out of the stables for pasture, feeding on quality hay, spraying or wetting hay used for feed, use of silage and haylage, pelleted and bracketed feed instead of hay, spraying grains with molasses to reduce the amount of dust in the feed, etc. Also, care should be taken on selecting bedding so that instead of poor-quality straw and hay, use of carton, paper, and specially treated wood shavings is recommended (Ainsworth and Biller, 1998; Robinson, 2001; Rose and Hodgson, 1993; Trailovic, 2000).

- The most common macroscopic finding in the studied horses combines emphysema and absence of lung collapse. Pathohistologically in all studied horses chronic bronchitis/bronchiolitis was evident with characteristic changes in the lumen, mucosa, fur, and smooth muscle layer.
- Alveolar emphysema was evident in 70.59% of the studied horses, more commonly as distensive emphysema (54.9%), and less commonly as destructive emphysema (15.69%).
- Increased immune reactivity in the subepithelial region of bronchioli and bronchus, in peribronchiolar and peribronchial tissues and less perivascularly, and in lung interstitium and alveolar septa suggest their importance in the development of COPD. Population of CD79+ lymphocytes was evidenced in the cortical region, in germinative centers of hyperplastic follicles, whereas the population of CD3+ lymphocytes was identified in the marginal region of the follicles and paracortex of the lymph nodes.
- The primary finding on cytological imprint preparations from the tracheal bifurcation is the thick, viscous, PAS-positive mucus that make curly Curschmann’s spirals; the predominant cellular population is composed of desquamated epithelial cells of the airways with the presence of eosinophilic and neutrophilic granulocytes, mastocytes, erythrocytes, and alveolar macrophages. This suggests the presence of asthmatic pattern analogous to that found in humans.
- *Streptococcus equi*, *Streptococcus pyogenes*, and *Staphylococcus aureus* were isolated from lungs of horses that suffered from pneumonia in addition to COPD; in addition to numerous pathogenic bacterial flora potential...
contaminants and causative organisms of secondary infections were also present—Enterococcus sp., Enterobacter sp., Citrobacter sp., Klebsiella sp., Proteus mirabilis, Pseudomonas sp., E. coli, and fungus Candida albicans.

- Pathohistological, cytological, immunohistochemical, and bacteriological findings are mutually correlated suggesting that chronic bronchitis/bronchiolitis is the main substrate of COPD in horses and that it has combined inflammatory and immune etiology, in which emphysema occurs secondarily, as a result of airway obstruction.

References


