

THESIS

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**COMPUTED TOMOGRAPHY BASED EXAMINATION OF THE
COMPLEX RESPIRATORY DISEASES OF SWINE**

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ABBREVIATIONS

ANOVA	analysis of variance
<i>A. pleuropneumoniae</i> (APP)	<i>Actinobacillus pleuropneumoniae</i>
<i>T. pyogenes</i>	<i>Trueperella pyogenes</i>
<i>A. suis</i>	<i>Actinobacillus suis</i>
<i>B. bronchiseptica</i> (Bb)	<i>Bordetella bronchiseptica</i>
CF	complement fixation
CT	computed tomography
ELISA	enzyme-linked immunosorbent assay
EM	electron microscopy
FA	direct fluorescent antibody assay
FAT	fluorescent antibody test
FB ₁	fumonisin B ₁ toxin
GLM	<i>general linear model</i>
<i>H. parasuis</i>	<i>Haemophilus parasuis</i>
HI	hemagglutination inhibition
HRCT	high resolution CT
HU	Hounsfield Unit
IFA	indirect fluorescent antibody assay
IHA	indirect hemagglutination
IHC	immunohistochemistry
IPMA	indirect immunoperoxidase monolayer assay
ISH	in-situ hybridization
IT	intratracheal infection through an endotracheal tube
LSD	least significant difference
<i>M. hyopneumoniae</i> (Mh)	<i>Mycoplasma hyopneumoniae</i>
<i>M. hyorhinis</i>	<i>Mycoplasma hyorhinis</i>
MIP	Medical Image Processing software
MRI	magnetic resonance imaging
NOAEL	no observed adverse effect level
<i>P. multocida</i> (Pm)	<i>Pasteurella multocida</i>
PCR	polymerase chain reaction
PCV	porcine circovirus
PDNS	porcine dermatitis and nephropathy syndrome

PMWS	postweaning multisystemic wasting syndrome
PMT	<i>Pasteurella multocida</i> toxin
PPE	porcine pulmonary oedema
PRCV	porcine respiratory coronavirus
PRDC	porcine respiratory disease complex
PRRSV	porcine reproductive and respiratory syndrome virus
RFLP	restriction fragment length polymorphism
<i>S. suis</i>	<i>Streptococcus suis</i>
SAS	<i>Statistical Analysis System</i>
SIV	swine influenza virus
SPF	specific pathogen free
SVN	serum-virus neutralization
TGEV	transmissible gastroenteritis coronavirus
VI	virus isolation

1. REVIEW OF THE LITERATURE

1.1. The porcine respiratory disease complex (PRDC)

The overwhelming majority of porcine respiratory diseases are disease entities developing in the simultaneous presence of multiple pathogens. In the case of these syndromes, the appearance of clinical signs and the magnitude of economic losses are decisively influenced by the predisposing factors (primarily the management, care and feeding conditions). Today, the multifactorial diseases belonging to this category are referred to in the special literature as porcine respiratory disease complex (PRDC) (Dee, 1996, Halbur, 1998, Thacker, 2001a). In connection with such disease entities, we can speak about obligate and opportunistic pathogens. Obligate pathogens can overcome the host's natural resistance and cause disease also on their own. However, opportunistic pathogens require predisposing factors, which may be adverse environmental effects or lesions caused by an obligate pathogen. The obligate pathogens include respiratory viruses (PRRS virus, influenza viruses, porcine circovirus type 2, Aujeszky's disease virus, porcine respiratory coronavirus) and bacteria (*Mycoplasma hyopneumoniae*, *Actinobacillus pleuropneumoniae*, *Bordetella bronchiseptica*). The opportunistic pathogens primarily include different bacteria (*Pasteurella multocida*, *Haemophilus parasuis*, *Streptococcus suis*, *Actinobacillus suis*, *Trueperella pyogenes*). Depending on the circumstances, certain opportunistic bacteria can behave like obligate pathogens (Brockmeier et al., 2002a).

In most pig herds affected by PRDC, the respiratory disease is due to a combination of one or two viruses, *M. hyopneumoniae* and several bacteria causing secondary infection. *M. hyopneumoniae* has long been regarded by experts as one of the most prevalent and economically most harmful pathogens responsible for porcine respiratory diseases. However, in the past two decades numerous new pathogens appeared and became known, which have acquired decisive importance in the aetiology of PRDC. At the end of the 1980s, the appearance and worldwide spread of PRRS virus brought major changes in the health status of pig herds. Subsequently, further pathogens playing an important role in the aetiology of PRDC became known, such as porcine circovirus type 2, porcine coronavirus and new strains of swine influenza viruses (e.g. H3N2), which also became widespread all over the world in the past 10–20 years and cause serious losses in countries having a well-developed pig production industry (Brockmeier et al., 2002a).

The past few decades have seen a rapid increase of productivity also in the pig production sector, accompanied by the size increase and concentration of pig herds and the spread of intensive management technologies. These changes, however, have brought along a drastic increase in the prevalence of respiratory diseases (Brockmeier et al., 2002a).

The so-called non-infectious predisposing factors resulting from the management technology and the adverse environmental effects substantially contribute to the development of respiratory diseases, either by increasing the chance of pathogen transmission or by raising the animals' stress level and damaging the respiratory organs. The most important environmental predisposing factors are the different nutritional anomalies, overcrowding, inadequate ventilation, extreme temperature conditions, commingling of pigs of different origin, insufficient downtime and disinfection between consecutive production groups, and inadequate animal health management. Overcrowding and inadequate ventilation lead to overheating or cooling as well as enhanced stress. Elevated dust and ammonia levels of the air in pig houses adversely affect the natural resistance of the respiratory organs. The commingling of pigs originating from different breeding herds and the continuous use of pig houses without proper disinfection and downtime between consecutive groups highly facilitate the spread of respiratory diseases (Brockmeier et al., 2002a).

Although intensive pig production implies the possibility of efficient infectious disease control, in large pig herds it sometimes occurs that a subpopulation of infected pigs develops and persists, transmitting pathogens to unprotected piglets in the period around weaning, when the piglets' passive protection derived from the colostrum wanes. Many respiratory pathogens are present in almost all pig herds, and on many pig farms a multitude of respiratory pathogens are circulating. It occurs even in the best managed pig herds that diseases are introduced with latently infected animals, causing severe disease outbreaks in naive animals, whose immune system has not yet been exposed to a given pathogen (Brockmeier et al., 2002a).

1.2. The most important respiratory viruses

1.2.1. Porcine reproductive and respiratory syndrome virus (PRRSV)

PRRS virus is a widely prevalent pathogen of pigs, which can cause not only respiratory but also reproductive disease. The disease was first observed and described at the end of the 1980s in the United States (Keffaber, 1989), while its first European occurrence was reported in Northwestern Germany (Lindhaus and Lindhaus, 1991).

Its presence in Hungary has been known since 1995 based upon the serological test results of Hornyák et al. (1996). PRRS virus belonging to the European genotype was first isolated in Hungary in 1999 (Medveczky et al., 2001).

According to experts, about 10% of the Hungarian pig herds can be considered infected with PRRSV. This ratio is much more favourable than that reported in countries west of Hungary, in some of which the prevalence of PRRSV exceeds 50% (Balka, 2009).

The results of clinical studies demonstrate that PRRS is often associated with disease outbreaks caused by other pathogens, and PRRS virus is nowadays one of the viruses most frequently isolated from cases of PRDC. Concomitant infection with *B. bronchiseptica* and PRRS virus highly predisposes pigs to secondary bacterial infections, especially to colonisation by *P. multocida* (Brockmeier et al., 2001). Concomitant infection by PRRS virus and *M. hyopneumoniae* was reported to cause pneumonia of increased severity (Thacker et al., 1999). There is evidence that PRRS virus interacts with other viruses causing respiratory disease, such as porcine coronavirus and swine influenza virus, and that it changes the typical course of disease caused by certain pathogens such as *H. parasuis* (van Reeth and Pensaert, 1994; van Reeth et al., 1996; Solano et al., 1997). In contrast, certain infection experiments using a combination of PRRS virus and other pathogens (*P. multocida*, *M. hyopneumoniae* and TGEV) did not show an increase in the occurrence and severity of clinical signs (Cooper et al., 1995; Carvalho et al., 1997; Wesley et al., 1998; Thacker et al., 1999; Brockmeier et al., 2001).

1.2.2. Swine influenza virus (SIV)

Swine influenza virus has been known for almost one hundred years. The disease was first observed in the United States in 1918 and became known as 'hog flu'; however, the influenza virus causing it was not detected until 1931. Up to the 1970s, it had occurred almost exclusively in the United States, but subsequently it became widespread in the pig herds of Europe (Nardelli et al., 1978) as well as Asia (Scholtissek et al., 1998; Varga, 1999). The subtype H1N1 became highly prevalent throughout North America, Europe and Asia. Before 1998, swine influenza was caused almost exclusively by the H1N1 subtype in America (Nfon et al., 2011). However, from the middle of 1998 the H3N2 subtype spread throughout the United States within a very short time (Zhou et al., 1999). Between 1998 and 2000, the H3N2 subtype was identified in more than 50% of the swine influenza cases diagnosed by the Iowa State University (Schneider and Yoon, 2001). Both subtypes are present in the European pig herds and cause acute outbreaks in the endemically infected countries from time to time. From the 1970s, with the spread of swine

influenza virus the acute respiratory disease outbreaks were most frequently due to swine influenza in the Netherlands and Belgium (van Reeth and Nauwynck, 2000; de Jong et al., 2001).

The third most prevalent subtype is H1N2, which was first isolated in the United Kingdom (Brown et al., 1995) but has since been described in the United States as well (Karasin et al., 2000).

Although swine influenza virus is often isolated from PRDC cases, its role in the pathogenesis of PRDC has not been clarified yet. The increased susceptibility of pigs to concomitant infections is perhaps due to the damage of the mucociliary apparatus and the impaired macrophage function (Brockmeier et al., 2002a). When acting in combination with other respiratory viruses, SIV can produce more severe disease. Simultaneous infection with PRRSV or *M. hyopneumoniae* resulted in respiratory disease of higher severity and longer duration (van Reeth et al., 1996; Thacker et al., 2001b). A case study reported that simultaneous infection with SIV and PCV-2 resulted in clinical signs and morbidity rates typical of acute SIV infection, but combined infection resulted in a disease of longer duration and associated with higher mortality in the herd (Harms et al., 2002).

1.2.3. Porcine circovirus (PCV)

Porcine circovirus type 2 (PCV2) is currently one of the pathogens causing the highest economic losses to the pig industry. One type of the diseases caused by PCV2, the circovirus-induced postweaning multisystemic wasting syndrome (PMWS) was first described in Canada in 1991 (Harding and Clark, 1996). By now the pathogen has become widespread all over the world. Since the appearance of PMWS, the number of diseases appearing to be related to PCV2 has increased considerably; therefore, today the term ‘PCV-related diseases’ is already more commonly used (Cságola, 2009). So far, PCV2 has been brought into connection with the following diseases, in addition to PMWS: PRDC (Halbur, 1998; Thacker, 2001a; Kim et al., 2003a), porcine dermatitis and nephropathy syndrome (PDNS) (Segalés et al., 1998a; Rosell et al., 2000), fetal myocarditis and reproductive disturbances (West et al., 1999; O’Connor et al., 2001), necrotic pneumonia (Pesch et al., 2000), necrotic tracheitis (Candotti et al., 2001), exudative epidermitis (Wattrang et al., 2002; Kim and Chae, 2004), and congenital tremor (Stevenson et al., 2001; Choi et al., 2002). With the exception of PMWS, PRDC, reproductive disorders and certain cases of PDNS, the causative role of PCV2 has not been proved in all cases. However, the presence of the virus indicates that it may participate in inducing the given disease entity, if not on its own, then at least as a concomitant infection.

1.2.4. Aujeszky's disease virus

Earlier, Aujeszky's disease virus was one of the most widespread pathogens in the pig herds. Because of the huge economic losses caused by the disease, after the appearance of vaccines of adequate efficacy several countries started eradication programmes to stamp out the disease. By now, most countries of the European Union as well as the domesticated pig population of the United States, Canada and New Zealand have already attained the disease-free status. In several countries (including Hungary) the procedure aimed at the official recognition of Aujeszky's disease free status is currently in progress. In countries where eradication of Aujeszky's disease has not been started yet, the disease causes a very severe problem to the pig producers even at present (OIE, 2012).

It is experimentally proven that Aujeszky's disease virus has synergistic effects with numerous bacteria (*A. pleuropneumoniae*, *P. multocida*, *S. suis*, *M. hyopneumoniae*, *H. parasuis*) and also with other viruses (PRRS virus), giving rise to diseases of increased severity (Fuentes and Pijoan, 1987; Iglesias et al., 1992b; Sakano et al., 1993; Narita et al., 1994; Shibata et al., 1998; de Bruin et al., 2000).

1.2.5. Porcine respiratory coronavirus (PRCV)

Porcine respiratory coronavirus is a deletion mutant variant of TGE virus and, thus, does not have the S gene present in TGE virus (Rasschaert et al., 1990; Laude et al., 1993). PRCV was first described in Europe in 1984 (Pensaert et al., 1986) and then in the United States in 1989 (Wesley et al., 1990). The spread of PRCV was much faster in Europe where it became enzootic in several countries within a short time (Laval et al., 1991; Groschup et al., 1993; van Reeth and Pensaert, 1994).

The role of this virus in the aetiology of PRDC is still being studied; however, this virus can commonly be isolated together with PRRS virus and/or swine influenza virus, enhancing the host's susceptibility to secondary bacterial infections (Lanza et al., 1992; van Reeth and Pensaert, 1994; van Reeth et al., 1996).

1.3. The most important respiratory bacteria

1.3.1. *Mycoplasma hyopneumoniae*

The structure of mycoplasmas is in several aspects markedly different from that typical of other bacteria. Among others, they contain only a small amount of genetic material and do not have a cell wall, being surrounded by a thin cytoplasmic membrane only. They are characterised by diversity in shape, and can have coccoid, lemon, pear or branching filamentous shape. They are Gram-negative and do not readily take up stains. They are microorganisms difficult to culture (Varga et al., 1999; Thacker, 2006).

Mycoplasma pneumoniae of pigs is a disease that often takes a chronic course and causes heavy economic losses. Its causative agent is *M. hyopneumoniae* that causes infection only in the pig. It was isolated from pigs suffering from respiratory disease in the 1960s, then the disease caused by it, which was to become known later as enzootic pneumonia of pigs, was successfully reproduced experimentally (Goodwin et al., 1965; Mare and Switzer, 1965). Subsequently, the outstanding pathological role of *M. hyopneumoniae* was demonstrated by numerous studies all over the world. This agent is present mainly in growing and fattening pigs, and gives rise to a health problem reducing the profitability of pig fattening (Brockmeier et al., 2002a; Thacker, 2006; Sibila et al., 2009). The route of infection is predominantly airborne, after which the mycoplasmas adhere to the epithelial cells of the airways with the help of their adhesin proteins. First they colonise the upper airways, then spreading deeper and deeper, they eventually permanently colonise the lower airways. The toxic metabolic products of mycoplasmas damage the epithelial cells of the airways, the cells become degenerated and denuded, losing their cilia, then they die and become detached from the surface of the mucous membrane. This severely impairs the functioning of the mucociliary apparatus and weakens the local immune defence of the respiratory tract mucosa (DeBey and Ross, 1994; Brockmeier et al., 2002a; Thacker, 2006). The inflammatory process developing around the site of infection is first characterised by cellular infiltration (with neutrophil granulocytes, monocytes, and lymphocytes), followed by increased epithelial cell proliferation. The cranioventral lung areas are the most severely affected: these are often atelectatic, of reddish and then of greyish colour, and compact and meaty to the touch. The mycoplasmas also damage the macrophages participating in the local immunity of the respiratory tract, resulting in local and generalised immunosuppression. As a consequence of this and the damage of the mucociliary apparatus, complicated disease forms accompanied by the proliferation of other pathogens can easily develop (Caruso and Ross, 1990; Varga et al., 1999; Brockmeier et al., 2002a; Thacker, 2004).

M. hyopneumoniae exerts a nonspecific stimulating (mitogenic) effect on lymphocytes (Messier and Ross, 1991). In addition, the production of proinflammatory cytokines plays an important role in pneumonia caused by *M. hyopneumoniae*, and it exerts an effect also on other pathogens present in the respiratory tract of piglets (Asai et al., 1993, 1994; Thacker et al., 2000).

Direct spread from animal to animal is the most important route of transmission involved in the epidemiology of mycoplasma pneumonia. Infection is most often spread by infected animals introduced into herds or groups of susceptible animals previously not exposed to the pathogen. The disease usually occurs in a massive form in the autumn and winter months, when the *ab ovo* not perfect environmental conditions tend to worsen further. Young animals are more susceptible to infection, and the disease spreads rather slowly, usually not manifesting itself in clinical signs before a few months of age. The disease often takes an asymptomatic course and its presence can be inferred from the poor performance parameters and the slaughterhouse findings only. The clinical signs of the above-mentioned different complications may be diverse, but most often dyspnoea and dry cough occur (Varga et al., 1999; Brockmeier et al., 2002a). Concomitant infection occurring in the presence of other pathogens is common; in this case, the spread of infection accelerates and the disease assumes a more severe form. Thus, the dead animals rarely show gross pathological changes typical of a pure mycoplasma pneumonia; deaths occur and necropsies are performed mainly in the complicated cases, which are therefore seen only during examination at the slaughterhouse in most cases (Brockmeier et al., 2002a; Choi et al., 2003; Hansen et al., 2010; Fablet et al., 2012).

M. hyopneumoniae has been experimentally shown to predispose pigs to infection by *A. pleuropneumoniae* and *P. multocida* infection (Yagiashi et al., 1984; Ciprian et al., 1988; Amass et al., 1994) and to increase the severity and duration of PRRSV-induced pneumonia (Thacker et al., 1999).

1.3.2. *Bordetella bronchiseptica*

B. bronchiseptica is a short rod-shaped, Gram-negative bacterium capable of active motility. The virulent strains produce numerous virulence factors, the most important of which are adhesins (filamentous haemagglutinin, pertactin, fimbriae) and toxins (dermonecrotxin, adenylate cyclase-haemolysin toxin, tracheal cytotoxin) (Magyar, 1999; Brockmeier et al., 2002a; Brockmeier et al., 2002b; Magyar et al., 2002).

Also when acting alone, *B. bronchiseptica* can produce pathological changes in the respiratory tract of numerous animal species and also of humans. In pigs, its aetiological role

played in the development of atrophic rhinitis was described first (Switzer, 1956). According to the present status of our knowledge, *B. bronchiseptica* acting alone produces a milder form of atrophic rhinitis. However, in combination with the toxic strains of *P. multocida* it plays a role in giving rise to a more severe and progressive form of that disease (Rutter and Rojas, 1982; Chanter et al., 1989). In the case of PRDC, *B. bronchiseptica* acts as a obligate pathogen facilitating the colonisation and enhancing the pathogenic effect of other bacteria and viruses, thus contributing to the production of also other, more severe diseases (Brockmeier et al., 2002a).

Colonisation by *B. bronchiseptica* occurs early, already in a few days old piglets. Adherence to the mucous membranes of the airways is facilitated by adhesins produced by the bacterium. The dermonecrotxin (Roop et al., 1987; Magyar, 1999) and the adenylate cyclase-haemolysin toxin (Brockmeier et al., 2002a) produced by the bacterium have a decisive role in the production of the characteristic clinical signs (sneezing, serous nasal discharge) and pathological changes (inflammation of the nasal mucosa, atrophy of the turbinate cartilages) as well as in facilitating colonisation by other pathogens (*P. multocida* in the case of atrophic rhinitis). However, colonisation by *B. bronchiseptica* and the severity of changes caused by it depend partly on the immune status of the dam (older and vaccinated sows and those having undergone a *B. bronchiseptica* infection previously transfer more antibodies to their piglets), and partly on the age of piglets exposed to the pathogen (the severity of changes markedly decreases with age) (Varga et al., 1999).

As a result of the pathological processes taking place in the upper and lower airways the mucous membrane of the airways will become hyperaemic, while the epithelial cells become damaged and lose their cilia. In the lungs, small haemorrhages due to injury of the walls of alveoli can be seen, together with leukocytic infiltration in the perialveolar and peribronchiolar tissues. Later on this is replaced by the proliferation and fibrosis of the epithelial cells (Brockmeier et al., 2002a).

B. bronchiseptica belongs to the pathogens frequently isolated from disease entities of the PRDC (Schöss and Alt, 1995; Runge et al., 1996; von Altrock, 1998; Palzer et al., 2007). Earlier experiments demonstrated that in young piglets *B. bronchiseptica* can produce pneumonia also when acting alone (Meyer and Beamer, 1973; Janetschke et al., 1977; Underdahl et al., 1982).

The disease entity occurring in the simultaneous presence of *B. bronchiseptica* and other respiratory pathogens is more severe than that induced by *B. bronchiseptica* alone (Brockmeier et al., 2000; Brockmeier et al., 2004; Brockmeier et al., 2008).

B. bronchiseptica has been demonstrated to enhance colonisation by, and/or exacerbation of the disease caused by *P. multocida*, *S. suis* and *H. parasuis* (Cowart et al., 1989; Vecht et al., 1992; Wesley et al., 1998; Brockmeier et al., 2001). Furthermore, while PRRSV or *B. bronchiseptica* alone does not increase susceptibility to pneumonia caused by *P. multocida*, the combination of the two already does (Brockmeier et al., 2001).

1.3.3. *Pasteurella multocida*

P. multocida is a Gram-negative bacterium of coccoid or short rod shape. It sometimes occurs that freshly isolated strains stain only at their two ends (bipolar staining). Most strains form a capsule and their colonies are viscous and mucinous. Serotyping is based on the capsular and cell wall antigens. Based on the capsular antigens, so far five serotypes (A, B, D, E and F), while according to the cell wall antigens 11 serotypes (by agglutination test) and 16 serotypes (by precipitation test) have been distinguished. Classification based upon the capsular antigens is more commonly used, and this form of classification is mentioned more often in the literature as well (Varga et al., 1999).

From mastitis cases *P. multocida* A strains (Pijoan et al., 1984; Cowart et al., 1989; Vena et al., 1991; Djordjevic et al., 1998; Davies et al., 2003; Ross, 2006; Palzer et al., 2008), while from cases of atrophic rhinitis serotype D strains are isolated more often, although both types have been recovered from both diseases (Pijoan et al., 1983; Kielstein, 1986). According to reports published in the literature, pure *P. multocida* infections usually cause respiratory diseases of mild course. However, in the previous presence of other pathogens and following the damage of the respiratory tract, *P. multocida* may cause respiratory organ changes of varying severity, with the corresponding clinical signs (Brockmeier et al., 2002a).

The toxin of protein nature, produced by certain strains of *P. multocida* (*P. multocida* toxin, PMT) is the primary virulence factor of this bacterium in atrophic rhinitis, when atrophy of the turbinates, deviation of the nasal septum and distortion of the nasofacial part of the head are attributable to the effects of this toxin. This was demonstrated in experiments in which the above-mentioned changes could be produced by administering the purified toxin into the airways, without the presence of the bacterium itself (Dominick and Riemler, 1986).

Prior colonisation by *B. bronchiseptica* is known to be a very important predisposing factor (van Diemen et al, 1994). The role played by PMT in the aetiology of pneumonia is not clarified yet. From the majority of pneumonia cases non-toxicogenic type A strains can be isolated (Varga et al., 1999; Brockmeier et al., 2002a).

Especially in the case of type A strains, the capsule may be an important virulence factor involved in the avoidance of phagocytosis (Fuentes and Pijoan, 1987). It is difficult to infect piglets experimentally with a pure *P. multocida* culture. Monoinfection is very rare in the practice; even if it occurs, it causes only very mild clinical signs and discrete changes in the respiratory apparatus (Bentley and Farrington, 1980; Hall et al., 1990; Ono et al., 2003). Numerous viruses and bacteria have been demonstrated to predispose to secondary infection by *P. multocida*. The clinical signs and pathological lesions occurring in such cases are usually more severe than what the obligate agent alone would cause. Chronic intermittent coughing, dyspnoea and growth retardation have been reported (Fuentes and Pijoan, 1987; Ciprián et al., 1988; Amass et al., 1994; Chung et al., 1994; Halloy et al., 2005).

Several studies have reported that *P. multocida* could be detected the most frequently from respiratory diseases due to mixed infections (Bentley and Farrington, 1980; Schöss and Alt, 1995; Runge et al., 1996; von Altrock, 1998; Palzer et al., 2007; Palzer et al., 2008).

1.3.4. *Actinobacillus pleuropneumoniae*

A. pleuropneumoniae is one of the most important pathogens involved in the aetiology of porcine pleuropneumonia. It occurs all over the world. The pathogen was first isolated in the 1960s in Great Britain, California and Argentina (Matthew and Pattison, 1961; Shope, 1964), and was designated as *Haemophilus pleuropneumoniae* (Shope, 1964). As a result of studies conducted in the 1980s, it was reclassified into the genus *Actinobacillus* under the name of *A. pleuropneumoniae* (Pohl et al., 1983; Kilian and Biberstein, 1984).

The complex effects of *A. pleuropneumoniae* and other respiratory pathogens on the respiratory organs have already been demonstrated in several experiments. In combination with PRRS virus (Pol et al., 1997), Aujeszky's disease virus (Sakano et al., 1993) and *M. hyopneumoniae* (Caruso and Ross, 1990), *A. pleuropneumoniae* causes more severe disease than alone, and the Apx toxins predispose pigs to infection by *P. multocida* (Chung et al., 1994).

1.3.5. *Haemophilus parasuis*

H. parasuis is a Gram-negative bacterium that causes fibrinous polyserositis, polyarthritis and meningitis (Glässer's disease) (Amano et al., 1994) or pneumonia (Little, 1970) in pigs.

Very often other pathogens can also be cultured from cases of pneumonia, and the type and severity of lesions depend on the presence of these pathogens (Rapp-Gabrielson et al., 2006). There have been attempts to reproduce pneumonia caused by *H. parasuis* experimentally; however, it appears that the presence of other pathogens, such as Aujeszky's disease virus (Narita et al., 1994) or PRRS virus (Solano et al., 1997; Solano et al., 1998; Segales et al., 1998b; Segales et al., 1999) is also needed for the development of pneumonia.

1.3.6. *Streptococcus suis*

S. suis is a Gram-positive bacterium carried by pigs in their tonsils and nasal cavity. It sporadically causes systemic and respiratory disease. Suckling piglets are infected by the sow very early; thus, the transmission cycle cannot be broken even by early weaning in the practice. At least 35 capsular serotypes of *S. suis* are known to exist. Capsular type 2 is the most common serotype, which can be isolated from affected pigs.

Like *H. parasuis*, *S. suis* is also frequently isolated from PRDC cases; however, induction of pneumonia with experimental *S. suis* infection is not typical, indicating that mixed infections can play a role in inducing the pathological processes taking place in the lungs. Certain studies have demonstrated that other respiratory pathogens, such as Aujeszky's disease virus (Iglesias et al., 1992b), PRRS virus (Halbur et al., 2000; Thanawongnuwech et al., 2000) and *B. bronchiseptica* (Vecht et al., 1989, 1992; Griffiths et al., 1991) predispose piglets to the disease caused by *S. suis*.

1.4. Other pathogens of minor importance

Pathogens less frequently isolated from PRDC cases include porcine cytomegalovirus, paramyxovirus, encephalomyocarditis virus, haemagglutinating encephalomyocarditis virus, adenovirus, *Salmonella choleraesuis*, *A. suis* and *T. pyogenes*; these pathogens may occur sporadically and in some places also endemically.

Table 1: Respiratory diseases in swine (Iowa State University Homepage, 2014)

Disease and etiologic agent	Clinical signs	Lesions gross - micro	Diagnostic aids/ comments
<p>Mycoplasma pneumoniae</p> <p><i>Mycoplasma hyopneumoniae</i></p>	<p>Persistent, dry cough, retarded growth, high morbidity/low mortality. Endemic in many herds.</p>	<p>Pneumonia and atelectasis in cranioventral lobules (gray-purple). Marked lymphoid hyperplasia around airways.</p>	<p>History and lesions are suggestive of diagnosis. FAT and IHC applied to lung tissue or PCR of airway swabs. Serology useful on a herd basis.</p>
<p>Atrophic rhinitis</p> <p><i>Bordetella bronchiseptica</i> and/or toxigenic <i>Pasteurella multocida</i></p>	<p>Sneezing, snorting, nasal discharge, epistaxis. Tear staining at medial canthi. Usually a deviation of snouts in a few pigs.</p>	<p>Variable turbinate atrophy. Perhaps deviation of snout. Tear staining. Secondary pneumonia often present.</p>	<p>Based on signs, history of reduced performance, typical lesions at necropsy or at slaughter. Culture and identify the agent(s) from turbinates or nasal swabs. Confirm toxigenicity.</p>
<p>Pneumonic pasteurellosis</p> <p><i>Pasteurella multocida</i></p>	<p>Coughing, dyspnea, fever, prostration.</p>	<p>Firm, fibrinous pneumonia cranioventrally and extending into diaphragmatic lobes. Variable pleuritis, perhaps adhesions/abscesses.</p>	<p>Culture <i>Pasteurella multocida</i> from lung lesions, possibly also from parenchymatous organs. Gray, firm, and subacute nature of lung lesions are suggestive of pasteurellosis. Organism a frequent, secondary, respiratory pathogen.</p>
<p>Pleuropneumonia (APP)</p> <p><i>Actinobacillus pleuropneumoniae</i></p>	<p>Acute: High temperatures, prostration, dyspnea, mouth breathing, perhaps bloody foam from nose. Short course, high morbidity and mortality. Chronic: chronic cough, unthriftiness</p>	<p>Pneumonia is firm, necrotic and bloody with fibrinous pleuritis. Chronic cases have pleural adhesions often with necrotic masses in lungs. Common in diaphragmatic lobes.</p>	<p>Signs and lesions are highly suggestive. Isolate agent from lung lesions and identify serotype. Serologically identify carrier herds by ELISA or CF tests.</p>
<p>Swine influenza (SIV)</p> <p>Influenza type A Subtypes H1N1 and H3N2 most common; diversity recognized.</p>	<p>Sudden onset, rapid spread of fever, serous nasal discharge, dyspnea, prostration. This is followed by loud coughing, and usually rapid recovery in seven days.</p>	<p>Multifocal to diffuse red firmness. Usually has lobular anteroventral distribution. Secondary bacterial pneumonia is common.</p>	<p>History and signs are very suggestive. Morbidity high, mortality low. Confirm presence of SIV by PCR or ELISA on nasal swabs or by PCR, IHC, FAT or VI from lung tissue. Serology by IHA or ELISA. Subtype determined by PCR.</p>

<p>Porcine reproductive and respiratory syndrome virus (PRRSV)</p> <p>Arterivirus</p>	<p>Respiratory disease and poor reproductive performance. High pre-weaning mortality. Respiratory dyspnea, fever and prolonged course, usually with abundant secondary agents.</p>	<p>In young pigs, focal or diffuse interstitial pneumonia in any or all lobes. Generally swollen lymph nodes. Perhaps secondary bronchopneumonia.</p>	<p>Chronic respiratory disease in growing swine. Identify virus in lung by IHC, FAT, or PCR. Isolate virus and identify. Demonstrate rising antibody titers in herd. Bronchoalveolar lavage is good specimen for PCR and isolation. Sequencing commonly performed by PCR.</p>
<p>Porcine Circovirus Type 2 (PCV2)</p> <p>Circovirus</p>	<p>These pigs have manifest ill thrift and labored respiration and often cough.</p>	<p>Granulomatous bronchointerstitial pneumonia with bronchiolitis and bronchiolar fibrosis, and abundant PCV2 antigen associated with the lesions is suggestive of PCV2-associated pneumonia and PRDC.</p>	<p>Characteristic lesions of PCV2-associated pneumonia. Detection of PCV2 nucleic acids (PCR, ISH). Detection of anti-PCV2 antibodies (SVN, IPMA, IFA, ELISA). Detection of PCV2 virus or viral antigen (VI, IHC, EM, IFA/FA, antigen-capture ELISA). Further characterization of PCV2 (RFLP, sequencing).</p>
<p>Pseudorabies (PRV)</p> <p>Herpesvirus</p>	<p>Central nervous system (CNS) signs predominate in neonates and the very young. (Sudden death common in very young) Swine >3 weeks old: sneezing, coughing, nasal discharge, dyspnea</p>	<p>Gross: often no lesions. Perhaps rhinitis, tonsillitis, tracheitis, keratoconjunctivitis.</p> <p>Histology: Nonsuppurative meningoencephalitis. Necrotic bronchitis, bronchiolitis, alveolitis.</p>	<p>Isolate and identify virus. Histopath with IHC. FAT on tonsil, spleen, brain. Serologic tests reveal herd infection. PRV was eradicated from US commercial swine in 2004. PRV should now be thought of as a foreign disease and all suspicious cases reported and investigated.</p>

1.5. Prevalence of different pathogens in PRDC

According to slaughterhouse data, the different types of pneumonia show 25–78% occurrence in the pig herds (Osborne et al., 1981; Wilson et al., 1986; Grest et al., 1997; Christensen and Enoe, 1999; Maes et al., 2001). The prevalence and incidence of pathogens playing a role in the aetiology of PRDC vary widely by country. The results of a large survey conducted at the Iowa State University in the United States between 1993 and 2000 support the presence of the above-mentioned most important respiratory pathogens in the respiratory diseases of pigs. According to the data reported at the end of the survey, in 2000, the most frequently isolated pathogens were the following: PRRS virus (in 1587 cases), *P. multocida* (in 971 cases), *M. hyopneumoniae* (in 823 cases), *S. suis* (in 639 cases), swine influenza virus (in 627 cases), porcine circovirus type 2 (in 456 cases), *A. pleuropneumoniae* (in 224 cases), *B. bronchiseptica* (in 206 cases), *S. choleraesuis* (in 177 cases), and *A. suis* (in 141 cases). The most important statement of that study was that during the 8 years of the survey there was a considerable increase in the incidence of PRRS virus (13-fold increase) and porcine circovirus type 2 (a 456-fold increase since the first isolation of the virus in 1996) in pigs affected by pneumonia (Brockmeier et al., 2002a). In 2000–2001, the lungs of a total of 2872 pigs affected by respiratory disease were examined for the presence of respiratory pathogens in the Veterinary Diagnostic Laboratory of the University of Minnesota. The results of that study were similar to those obtained during the Iowa survey with regard to the prevalence of pathogens. From the lung samples, the different pathogens were detected with the following frequencies: PRRS virus (35.4%), *P. multocida* (31.6%), *M. hyopneumoniae* (27.0%), swine influenza virus (22.2%, of that 50.1% H3N2, 47.8% H1N1 and 1.7% H1N2), *H. parasuis* (22.0%), porcine circovirus type 2 (18.6%), *A. pleuropneumoniae* (18.2%), *B. bronchiseptica* (8.0%), and porcine coronavirus (1.5%). Two or more pathogens were present in the same sample in 88.2% of the samples examined. PRRS virus occurred alone in 3.3% of the cases, while in the case of concomitant infections it was detected together with *P. multocida* (10.4%), *M. hyopneumoniae* (7.0%), *A. pleuropneumoniae* (6.2%), *H. parasuis* (6.1%), swine influenza virus (3.8%), and porcine circovirus type 2 (2.3%). Swine influenza virus alone was detected in 3.1% of the cases, but this agent also occurred more frequently together with other pathogens: with *P. multocida* (5.2%), *M. hyopneumoniae* (4.3%), PRRS virus (3.8%), and porcine circovirus type 2 (1.9%) (Choi et al., 2003).

In a Danish study, in which 148 pneumonic pigs were examined at two slaughterhouses, the most frequently detected pathogens were PCV-2, *M. hyopneumoniae*, *Mycoplasma hyorhinis* and *P. multocida* (Hansen et al., 2010).

In France, the incidence of pneumonia and the presence of different bacterial respiratory pathogens were studied in a survey involving 3731 pigs of 125 pig herds. The pathogens most frequently detected from pneumonic pigs were *M. hyopneumoniae* (69.3%), *P. multocida* (36.9%), *A. pleuropneumoniae* (20.7%), *S. suis* (6.4%) and *H. parasuis* (0.99%) (Fablet et al., 2012).

In a study involving 44 pig farms, conducted in Spain between 2000 and 2005, a 80–85% prevalence of PRRS and swine influenza was found, while the prevalence of Aujeszky's disease virus decreased from 41% to 30% during the period studied (López-Soria et al., 2010).

In South Korea, in a total of 105 PRDC cases PCV-2 was found in 85 cases, PRRSV in 66 cases and swine influenza virus in 14 cases; concomitant infection by more than one agent was found in 85 out of the 105 cases. Besides PCV-2, *P. multocida* (in 38 cases) and *M. hyopneumoniae* (in 33 cases) were isolated most frequently (Kim et al., 2003b).

In Germany, Palzer et al. (2008) studied pigs suffering from pneumonia (n = 239), from the bronchoalveolar lavage fluid of which *M. hyopneumoniae*, *M. hyorhinis*, PRRS virus, PCV-2, influenza virus type A, alpha-haemolytic *Streptococcus* species, beta-haemolytic *Streptococcus* species, *P. multocida*, *B. bronchiseptica*, *H. parasuis* and *A. pleuropneumoniae* could be isolated. In most cases, these pathogens occurred in some combination, and only rarely were they detected alone, in monoinfection. In pigs not showing clinical signs (n = 100) PCV-2 and alpha-haemolytic streptococci were detected.

In Slovakia, Holko et al. (2004) conducted a study in which respiratory pathogens were isolated from 98 pigs that had died among respiratory signs. *P. multocida* was isolated in 41 cases (44%), *A. pleuropneumoniae* in 38 cases (40.8%) and *M. hyopneumoniae* in 27 cases (29%). In five cases all three pathogens could be detected, but the most frequent concomitant infection was *M. hyopneumoniae* combined with *P. multocida* (Holko et al., 2004).

Monitoring studies have been conducted in several countries to determine the prevalence of the most important respiratory and other pathogens of domestic pigs in the wild boar population. The serological tests have confirmed that porcine circovirus type 2 has rather high prevalence in the wild boar population of the United States and also of some European countries: its prevalence was 59% in some hunting areas of North Carolina (Sandfos et al., 2012), 48% in Spain (Boadella et al., 2012), 43% in the Czech Republic (Sedlak et al. 2008) and 16.0% in Germany (Sattler et al., 2012); in addition, the prevalence of Aujeszky's disease virus, swine influenza virus and PRRS virus may also be remarkably high, although it varies by country.

1.6. Mycotoxins as environmental predisposing factors

Microscopic filamentous fungi contaminating feed and food raw materials produce numerous toxins (mycotoxins) which enter the soil–plant–animal–human food chain and pose a major public health risk. In addition to this, mycotoxins cause huge economic losses to the animal production sector. The number of toxic fungal metabolites known at present is more than one thousand, but newly discovered mycotoxins are continuously being added to the diverse array of these compounds. So far, about 100 mycotoxins have been demonstrated to exert adverse effects on living organisms, but according to our current state of knowledge only 15–20 mycotoxins have outstanding human and animal health importance (Rafai, 2003).

In the 1950s, the development of an until then unknown pulmonary oedema of pigs was regularly observed in Hungary, especially after the feeding of newly harvested maize; the disease showed massive occurrence in the affected herds. At that time the disease was described by Domán (1952) and Petrás (1952), and the entity became known as the ‘fattening or unique pulmonary oedema of pigs’.

Fumonisin (FB₁, FB₂, FB₃, FB₄) constitute a group of mycotoxins that was discovered and identified relatively recently, in 1988 (Gelderblom et al., 1988). In experiments with horses, fumonisin B₁ (FB₁) induced encephalitis and cerebral atrophy (encephalomalacia) (Marasas et al., 1988).

The mycotoxin FB₁ causes degeneration of the liver and kidney as well as pulmonary oedema in pigs (Harrison et al., 1990). It disturbs the homeostasis of cells and induces cell death, apoptosis (Wang et al., 1991). After the horse, the pig is the second most sensitive animal species to the mycotoxin FB₁.

The natural occurrence of fumonisins was first reported in the United States, in maize samples brought into connection with equine encephalomalacia (Norred et al., 1989), while that of FB₁ was first detected in South Africa, in maize originating from Transkei province (Sydenham et al., 1990), where the prevalence of human oesophageal cancer was very high. In Hungary, the first survey aimed at determining the FB₁ content of maize samples was started by Fazekas and his colleagues at the Veterinary Institute of Debrecen, in the middle of the 1990s. They determined the FB₁ content of mouldy maize samples collected from arable land, from ‘average’ maize samples collected at harvest, from pig and poultry diets, as well as from maize flour and maize meal samples prepared for human consumption. According to the results obtained, between 1993 and 1996 FB₁ occurred in about 70% of the mouldy samples, in an average concentration of 2.60–8.65 mg/kg, depending on the vegetation year (minimum value 0.05 – maximum value 75.10 mg/kg).

The mean FB₁ content of average maize samples was 0.1–1.6 mg/kg (minimum value 0.05 – maximum value 7.0 mg/kg). The mean FB₁ concentration measured in pig and poultry diets was 0.3–0.6 mg/kg. In maize products intended for human consumption, FB₁ was found only in trace amounts (0.016–0.058 mg/kg) (Fazekas et al., 1998).

FB₁ contamination was equally high in samples collected during the storage and the harvesting of maize, indicating that a substantial proportion of FB₁ is produced during the vegetation period of maize (Fazekas et al., 1998). In addition, it should be emphasised that the highest risk of FB₁ contamination is posed by maize stored in an obsolete manner (in a shed or on the cob) as well as by the maize tailings separated during sieving after combine harvesting (Fazekas et al., 1998).

Research aimed at obtaining a more comprehensive understanding of these pathological processes were started after American authors had first described the disease entity termed ‘porcine pulmonary oedema’ (PPE) of pigs, proven to be induced by the mycotoxin FB₁, together with the most important lesions related to it (pulmonary oedema, liver and kidney degeneration) (Harrison et al., 1990).

In Hungary, the first report on the results of FB₁ feeding experiments in pigs was published in 1997 (Fazekas et al, 1998).

The Animal Breeding and Animal Hygiene Research Group of the Hungarian Academy of Sciences and Kaposvár University conducted several experiments to study the changes caused by FB₁ toxin in pigs depending on the dose and the exposure time, to determine the still tolerable FB₁ limits and the ‘no observed effect level’ (NOAEL) (Zomborszky-Kovács et al., 2000, 2002). They have demonstrated pathological changes of acute pulmonary oedema not manifested in clinical signs that FB₁ toxin causes already at a low dose (5–10 mg/kg of feed); in addition, they demonstrated the chronic pulmonary fibrosis causing effect of low-dose (5–10 mg/kg of feed) FB₁ toxicosis and determined the pathogenesis of the disease which was accompanied by liver, kidney and myocardial damage in some cases. Based on these results, the maximum allowable limit of FB₁ was determined and declared as 5 mg/kg first in the Hungarian Feed Code (Codex Pabularis Hungaricus) and subsequently in the recommendation of the European Union (Commission Recommendation, 2006).

So far, few studies have been conducted to determine the predisposing effect of fumonisin B₁ toxin for diseases caused by the most important porcine respiratory pathogens. Halloy et al. (2005) demonstrated that in the presence of FB₁ toxin *P. multocida* serotype A induced a more severe and more extensive pneumonia than it did alone, unassisted by exposure to FB₁.

The immunosuppressive effect of FB₁ toxin might be related to the accumulation of free sphingoid bases which inhibits lymphocyte proliferation (Taranu et al., 2005).

Oswald et al. (2003) have shown that FB₁ taken up with the feed reduced natural resistance to *Escherichia coli*, resulting in enhanced colonisation of the small intestine by these bacteria. Stoev et al. (2012) found reduced antibody production in response to vaccination against Aujeszky's disease in pigs treated with the FB₁ mycotoxin.

Similarly, Halloy et al. (2005) assumed that the effect of FB₁ predisposing pigs for *P. multocida* infection was based on the impaired immune response and reduced phagocytosing ability of the pulmonary macrophages. Increased sensitivity of the pulmonary capillaries to FB₁ infection may also make the lungs more susceptible to infection (Halloy et al., 2005). Several studies have confirmed that this mycotoxin has a proinflammatory action as well (Taranu et al., 2005).

Ramos et al. (2010) could also demonstrate the predisposing effect of FB₁ toxin in pigs experimentally infected with PRRS virus and treated with FB₁ toxin, and also attributed it to the immunosuppressive effect of the toxin.

1.7. Use of computed tomography (CT) for examination of the lungs in humans and animals

1.7.1. Development of CT imaging

In 1917, the mathematician J. H. Radon gave evidence that the distribution of a material in an object layer can be calculated depending on the integral values along any number of lines passing through the same layer are known (Radon, 1917). The first application of Radon's reconstruction mathematics was carried out by Bracewell (Bracewell, 1956). The first successful practical implementation was established by an English engineer G. N. Hounsfield in 1972, now generally known as the inventor of computed tomography. In 1979 Hounsfield and Cormack, a physicist, were awarded the Nobel Prize for medicine for their outstanding achievements. The development of CT scanners started with Hounsfield's experimental set-up, which was termed the „first generation” of CT. The first commercial scanners, the so-called „second generation”, differed only slightly from Hounsfield's scanning system. To speed up scanning and to utilize the available x-ray power more efficiently detectors were added to entail going from a pencil beam to a small fan beam.

The translatory motion became obsolete, and the systems executed a rotatory motion only. The first whole-body scanners with fan beam systems were launched in 1976 providing scan times of 20 s per image. In the first scanners of this type both the x-ray tube and the detector rotated around the patient. This concept was called „third generation” CT scanner. Only a little later scanners followed with a ring-like stationary detector fully encircling the patient so that only the x-ray tube rotated; which was termed the „fourth generation” CT. The translation-rotation systems vanished entirely. The type of rotation system of the „third generation” turned to be more effective. Thus today, the so-called third-generation CT's became standard (i.e. conventional) (Kalender, 2006).

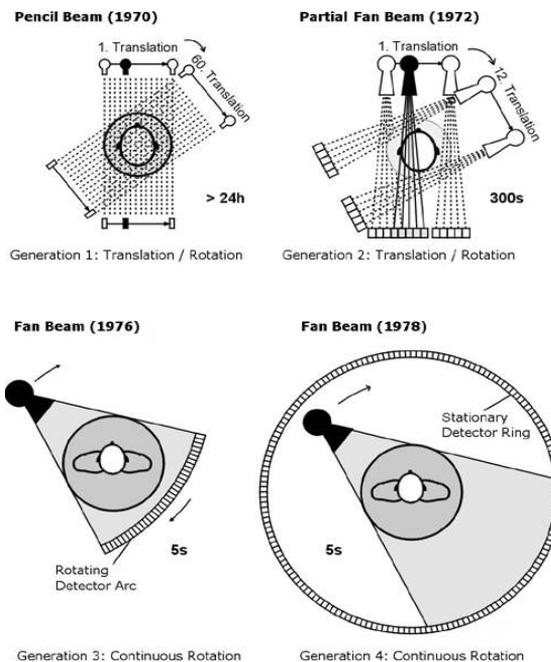


Figure 1: Four scanner generations were promoted in the 1970s (Kalender, 2006).

In these, the patient is scanned one slice at a time. The X-ray tube and detectors rotate for 360 degrees or less to scan one slice while the table and patient remain stationary. This slice-by-slice scanning is time-consuming, consequently efforts were made to increase the scanning speed. After the great technological progress in the 1970s, new achievement was seen in the start of 1990s, when the spiral/helical CT scanners were invented (Kalender, 1990). The novelty of this invention is that the X-ray beam traces a path around the patient (Kalender, 2006).

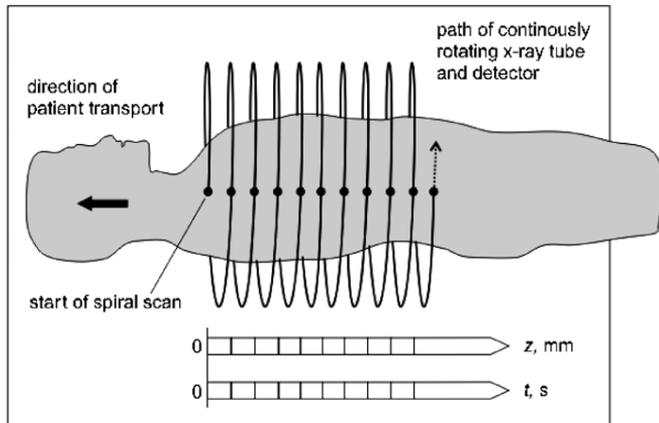


Figure 2: The „Spiral CT” (Kalender, 2006).

In 1998, all major CT manufacturers introduced multi-slice CT (MSCT) systems. It typically offered simultaneous acquisition of 4 slices at a rotation time of down to 0.5 s.

This was a significant development in scanning speed and longitudinal resolution and offered better utilization of the available X-ray power (Klingenbeck et al., 1999, McCollough and Zink, 1999, Ohnesorge et al., 1999, Hu, 2000). These improvements were quickly recognized as revolutionary developments that would finally enable users to do real isotropic 3D imaging (Kalender, 2006).

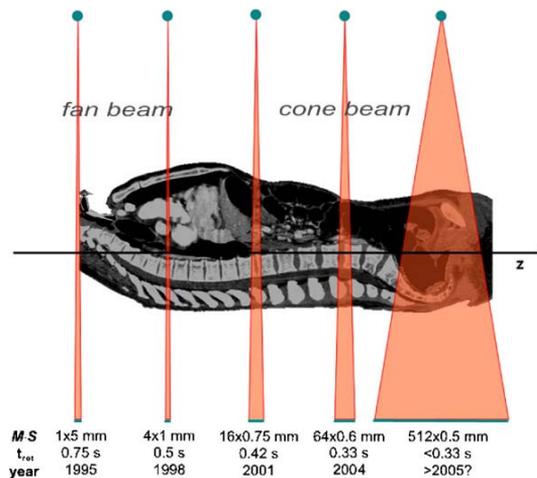


Figure 3: The development from fan-beam to cone-beam CT in the early 2000s (Kalender, 2006).

Volume scanning has resulted in the routine application of such advanced techniques as CT fluoroscopy, CT angiography, three-dimensional imaging and virtual reality imaging. Its main applications are in cardiovascular studies, functional imaging, trauma and oncology. In cardiology, gated studies with the multi-slice scanners can provide clear non-invasive images of the heart and its major vessels, as well as fast coronary artery imaging including distal segments and multiple branches. The speed and precision of the multi-slice scanners has also changed the approach to the diagnosis and treatment of cancer. Instead of just studying the morphology of a tumor and monitoring changes in size, it is now possible to follow the perfusion of a contrast agent through and around the tumor, which allows early information on the response to therapy. Other emerging applications for multi-slice scanners provide evaluation of carotid artery plaque, diagnostic of pulmonary diseases, and low-dose pediatric applications (Imhof, 2006).

Pixels in an image obtained by CT scanning are displayed in terms of relative radiodensity. Each pixel is assigned a numerical value (CT number), that is the average of all the attenuation values pertained within the corresponding voxel. This number is compared to the attenuation value of water and displayed on a scale of arbitrary units named Hounsfield units (HU) after Sir Godfrey Hounsfield. This scale assigns water as an attenuation value (HU) of zero. The range of CT numbers is 2000 HU wide although modern scanners have a greater range of HU up to 4000.

Each numeral value represents a shade of grey with +1000 (white) and -1000 (black) at either end of the spectrum. The "*Partial Volume Effect*" means the phenomenon that one part of the detector cannot differentiate between different tissues. This is typically done via a process of "windowing", which maps a range (the "window") of pixel values to a greyscale ramp. The X-ray density of the object of interest determines the window used for display in order to optimize the visible detail. The „lung windows“ typically have a window mean of approximately -600 to -700 HU and a window width of 1000 to 1500 HU. Lung windows best demonstrate lung anatomy and pathology, contrasting soft-tissue structures with surrounding air-field lung parenchyma. For example, CT images of the lung are commonly viewed with a window extending from -1100 HU to -100 HU. Pixel values of -1100 and lower, are displayed as black; values of -100 and higher are displayed as white; values within the window are displayed as a grey intensity proportional to position within the window. The window used for display must be matched to the X-ray density of the object of interest, in order to optimize the visible detail (Webb et al., 2014).

1.7.2. The lung CT imaging

The initial imaging tool for the lung parenchyma remains the chest radiograph. It is unsurpassed in the amount of information it yields as far as its cost, radiation dose, availability, and ease of performance. However, the chest radiograph has its limitations. In several studies, the chest radiograph has been shown to have an overall sensitivity of 80 percent and a specificity of 82 percent for detection of diffuse lung disease. Chest radiography could provide a confident diagnosis in only 23 percent of cases, and those confident diagnoses proved correct only in 77 percent of cases (Mathieson et al., 1989).

Advances in CT scanner technology have made isotropic volumetric, multiplanar high-resolution lung imaging possible in a single breath-hold, an essential advance over the incremental high-resolution CT (HRCT) technique in which noncontiguous images sampled the lung, but lacked anatomic continuity. HRCT of the lungs is an established imaging technique for the diagnosis and management of interstitial lung disease, emphysema, and small airway disease, providing a noninvasive detailed evaluation of the lung parenchyma, and providing information about the lungs as a whole and focally (Sundaram et al., 2010). HRCT, which has a sensitivity of 95 percent and a specificity approaching 100 percent, can often provide more information than either chest radiography or conventional CT scanning. A confident diagnosis is possible in roughly one-half of cases, and these are proven correct an estimated 93 percent of the time (Mathieson et al., 1989). The technique of HRCT was developed with relatively slow CT scanners, which did not make use of multi-detector (MDCT) technology. The parameters of scan duration, z-axis resolution and coverage were interdependent. To cover the chest in a reasonable time period with a conventional chest CT scan required thick sections (e.g. 10mm) to ensure contiguous coverage. As performing contiguous thin sections required unacceptably prolonged scan time, HRCT examination was performed with widely spaced sections. Because of the different scan parameters for conventional and HRCT examinations, if a patient required both, they had to be performed sequentially. Modern MDCT scanners are able to overcome this interdependence, and are capable of imaging at full resolution yet retain very fast coverage - images can then be reconstructed retrospectively from the volumetric raw data (Dodd et al., 2006).

1.7.3. Interpretation of lung disorders (Smithuis et al., 2006)

Reticular pattern

In the reticular pattern there are too many lines, either as a result of thickening of the interlobular septa or as a result of fibrosis.

Nodular pattern

In most cases small nodules can be placed into one of three categories: perilymphatic, centrilobular or random distribution.

Low Attenuation pattern

The fourth pattern includes abnormalities that result in decreased lung attenuation or air-filled lesions. These include: emphysema, lung cysts, bronchiectasia, honeycombing.

High Attenuation pattern

Moderate increased lung attenuation is called ground-glass-opacity (GGO) if there is a hazy increase in lung opacity without obscuration of underlying vessels. The more increased lung attenuation is called consolidation if the increase in lung opacity obscures the vessels. In both ground glass and consolidation the increase in lung density is the result of replacement of air in the alveoli by fluid, cells or fibrosis.

Ground-glass-opacity (GGO)

In GGO the density of the intrabronchial air appears darker as the air in the surrounding alveoli. Ground-glass opacity (GGO) represents: A) Filling of the alveolar spaces with pus, edema, hemorrhage, inflammation or tumor cells; B) Thickening of the interstitium or alveolar walls below the spatial resolution of the CT as seen in fibrosis.

So ground-glass opacification may either be the result of air space disease (filling of the alveoli) or interstitial lung disease (i.e. fibrosis).

Consolidation

Consolidation is synonymous with airspace disease. Is it pus, edema, blood or tumor cells.

Acute consolidation is seen in: pneumonias (bacterial, mycoplasmal), pulmonary edema, hemorrhage, acute eosinophilic pneumonia. Chronic consolidation is seen in: organizing pneumonia, chronic eosinophilic pneumonia, fibrosis, bronchoalveolar carcinoma or lymphoma.

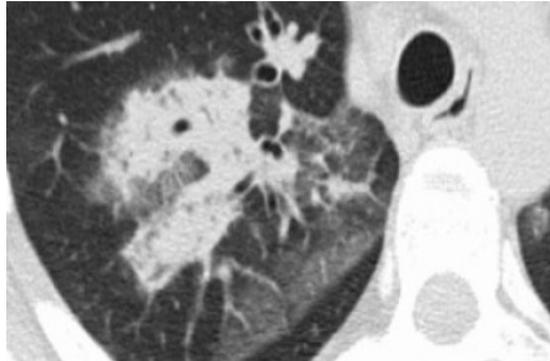


Figure 4: Broncho-alveolar cell carcinoma with ground-glass opacity and consolidation

Mosaic attenuation

The term mosaic attenuation is used to describe density differences between affected and non-affected lung areas. There are patchy areas of black and white lung. When ground glass opacity presents as mosaic attenuation consider: A) infiltrative process adjacent to normal lung; B) normal lung appearing relatively dense adjacent to lung with air-trapping; C) Hyperperfused lung adjacent to oligemic lung due to chronic thromboembolic disease

Crazy Paving

Crazy Paving is a combination of ground glass opacity with superimposed septal thickening. It was first thought to be specific for alveolar proteinosis, but later was also seen in other diseases.

Crazy Paving can also be seen in: infection (viral, mycoplasmal, bacterial), pulmonary hemorrhage, edema.

2. CONCLUSIONS DRAWN FROM THE DATA OF THE LITERATURE

Over the past few decades, there has been a very pronounced increase in productivity also in the pig industry, brought about by the size increase and concentration of pig herds and by the spread of intensive management technologies. This productivity increase, however, was accompanied by a drastic rise in the prevalence of respiratory diseases, among other changes. The vast majority of respiratory diseases often affecting large numbers of animals represent syndromes of multifactorial aetiology induced by the simultaneous presence of multiple pathogens, which are now collectively termed as porcine respiratory disease complex (PRDC) in the special literature.

Mycoplasma hyopneumoniae is one of the most prevalent respiratory pathogens. It has played a decisive role in the aetiology of respiratory diseases for several decades, usually in association with other pathogenic microorganisms, thus resulting in a complex respiratory syndrome. The prevalence of the other respiratory pathogens varies considerably; however, in the past two decades numerous new pathogens appeared or became known, which have assumed decisive importance in the development of PRDC. The appearance and worldwide spread of PRRS virus at the end of the 1980s brought major changes in the health status of pig herds. Subsequently, further pathogens playing an important part in the aetiology of PRDC became known, such as porcine circovirus type 2, porcine coronavirus and new strains of swine influenza virus (e.g. H3N2), which also spread all over the world in the past 10–20 years and are now causing major economic losses in the pig-producing countries.

Atrophic rhinitis is also a long-known and widely distributed pig disease, in the aetiology of which the interaction between toxigenic strains of two pathogenic bacteria, *Bordetella bronchiseptica* and *Pasteurella multocida*, is thought to play the primary role. These two pathogens are frequently isolated from the respiratory tract of pigs and they play a major role in the development of PRDC. Based on the data of the literature, the toxigenic strains of *P. multocida* are more often found in lesions restricted to the upper airways, whereas in the pathological processes taking place in the lungs mostly *P. multocida* strains not capable of toxin production are involved.

Microscopic filamentous fungi contaminating feed and food raw materials produce numerous toxins (mycotoxins) which, when entering the soil-plant-animal-human food chain, are a source of major public health risks. In addition, they cause substantial economic losses in the animal production sector. Fumonisin (FB₁, FB₂, FB₃, FB₄) constitute a relatively recently identified group of mycotoxins, which was discovered in 1988.

After American authors had first described the syndrome termed porcine pulmonary oedema (PPE), confirmed to be attributable to fumonisin B₁ (FB₁) toxin, together with the main pathological lesions associated with it (pulmonary oedema, liver and renal degeneration), research studies aimed at getting a closer insight into the pathological processes were started.

Hungarian researchers have achieved significant results in the study of disease entities caused by FB₁ toxin. It was on the basis of these results that the maximum permissible limit for FB₁ (5 mg/kg) was established and incorporated into the Codex Pabularis Hungaricus (the Hungarian Feed Code) and the relevant recommendations of the European Union.

To date, few studies have been done to determine the potential predisposing role of FB₁ toxin in syndromes induced by the most important porcine respiratory pathogens. The interaction between this mycotoxin and the above-mentioned pathogens was studied in pigs experimentally infected with serotype A of *P. multocida* and treated with FB₁ toxin (Halloy et al., 2005), as well as in pigs experimentally infected with PRRS virus and treated with FB₁ toxin (Ramos et al., 2010).

3. ANTECEDENTS AND OBJECTIVES OF THE DISSERTATION

At the Department of Animal Physiology and Hygiene of Kaposvár University, in co-operation with the Institute of Diagnostic and Oncoradiology of the same University, animal experiments aimed at following the pathological processes that take place in the lungs of pigs have been conducted since 1997. During these experiments, the basic techniques of using modern diagnostic imaging procedures for the above purpose were developed and applied.

Co-operation with the Institute for Veterinary Medical Research, Centre for Agricultural Research, Hungarian Academy of Sciences dates back to 2000. In its framework the impact of porcine atrophic rhinitis on production was studied and the pathogenesis of the disease was monitored by CT. A specific pathogen free (SPF) piglet-rearing method has been developed, by which freedom from *B. bronchiseptica* and *P. multocida* can be achieved. The method is suitable for performing infection experiments that require animals not infected by the given pathogens.

To date, only few studies have been done to determine the potential predisposing role of FB₁ in syndromes induced by the most important swine respiratory pathogens. Also, there is only scant literature on lung examinations using radiography and modern imaging modalities with the objective to monitor swine respiratory diseases.

The scientific work forming the basis of the present doctoral thesis was planned as a continuation of the earlier successful co-operation mentioned above.

Our principal objective was to obtain, by the use of CT as a modern diagnostic imaging modality, new data on the pathogenesis, pathological features, gross pathological lesions and histopathological changes of some common swine respiratory diseases of infectious origin, as well as on the predisposing and presumably pathogenesis-modifying role played by the FB₁ mycotoxin in these processes. We selected three respiratory pathogens for the model experiments: *B. bronchiseptica*, a toxigenic strain of *P. multocida* and *M. hyopneumoniae*.

An additional objective was to develop further the use of the diagnostic imaging modality in the study of swine respiratory diseases, including the elaboration of a numerical model that could facilitate the accurate evaluation of pathological processes taking place in the lungs of pigs.

4. METHODOLOGICAL SUMMARY OF THE DISSERTATION

In our experiments, we studied the interaction of certain bacteria playing a key role in the aetiology of PRDC with the mycotoxin FB₁ in inducing respiratory disease in swine. For these studies, we used piglets reared free from specific respiratory pathogens. A certain proportion of these piglets were infected experimentally and fed a diet contaminated with FB₁ toxin.

4.1. Artificial rearing of piglets

In selecting the herd of origin of piglets included in the experiment, basic criteria were the high animal health status and the absence or only minimal presence of major respiratory pathogens (Hungaro-Seghers Ltd., Mohács, Hungary). One week before farrowing the selected sows received antibiotic treatment (tulathromycin 100 mg/ml – Draxxin inj., Pfizer Inc., New York, USA) in a dose of 1 ml/40 kg of body weight. In addition, tests were performed for the presence of *P. multocida*, *B. bronchiseptica* and *M. hyopneumoniae*. The sows proved to be free from *P. multocida*. By serological tests, all selected sows had antibody titres of <1:64 for *B. bronchiseptica* and *M. hyopneumoniae*. All selected sows farrowed within an interval of maximum 24 hours. The piglets were left with their dams for 2 days, during which they could take up colostrum. Before transportation, the piglets were weighed and marked individually.

The piglets were transported to the Department of Animal Nutrition, Faculty of Agricultural and Environmental Sciences of Kaposvár University, where they were housed in two separate rooms prepared in advance, equipped with elevated-level battery cages (type M-10, Pig-Techn Ltd., Mohács, Hungary) and disinfected with aerosol fogging (KRKA d. d. / Antec International Ltd., Novo Mesto, Slovenia). The identical temperature of the rooms (27 °C) was adjusted with thermostat-regulated central heating, and adequate exchange of air was ensured by the use of exhaust fans. The battery cages, the drinkers and the rooms were cleaned twice a day (in the morning and in the evening). The piglet-rearing equipment was dismantled and cleaned every second day. Animal tenders entering the rooms wore protective clothing (overalls, rubber boots, rubber gloves, nose and mouth masks and caps) and disinfected their hands and feet with an aqueous solution of Virkon S (KRKA d. d./Antec International Ltd., Novo Mesto, Slovenia) when entering and leaving the rooms.

From the time of their arrival up to day 14 of the experiment, the piglets fed a milk replacer diet from a ‘Mambo’ automatic feeder which included a feeding unit with a plastic casing (consisting of a milk replacer tank, a milk replacer mixing unit operated by an electric motor, and a feeding tray) and an automatic control unit (mixing duration with a second scale and mixing frequency with an hour scale). The automatic equipment can be connected to both the electric mains and the water system. According to the adjustments of the automatic control unit, the screw conveyor operated by an electric motor dosed out the milk replacer diet from the storage tank into a specially designed mixing space. The preheated water jet entering the mixing space was mixed homogeneously with the milk powder, then was forwarded through a tube onto the feeding tray. The feeding tray is divided into 14 sections, thus providing convenient access for 14 piglets at the same time.

The piglets were fed a milk replacer diet (Salvana Ferkel Ammen Milch, Salvana Tiernahrung GmbH) from the time of their arrival (from 2–3 days of age) up to 21 days of age. The automatic feeder was calibrated to dissolve 100 g milk powder in 1 litre of preheated water. From 7 days of age a dry coarse meal (Salvana Pre-meal, Salvana Tiernahrung GmbH) was also given to the piglets *ad libitum*, and then from day 14 to the end of the experiment only the dry coarse meal was available to them.

Drinking water was provided from nipple drinkers (two drinkers per battery cage), and initially this was complemented with water offered from plastic drinking bowls. On the day after their arrival, the piglets were given iron and vitamin supplementation per os once (Bio-Weyx-In FeVit emulsion, Veyx-Pharma GmbH) in a dose of 2 ml/piglet).

4.2. Experimental design

The experimental series consisted of three separate experiments.

In **Experiment 1** a single-pathogen infection based on *B. bronchiseptica* was used, without FB₁ toxin treatment, in order to demonstrate and monitor the development of pneumonia induced by the pathogen in young piglets. The methods of CT studies were also elaborated during this experiment.

In **Experiment 2** we demonstrated and studied the lung lesions induced in young pigs by the interaction of two pathogens (*B. bronchiseptica* and *P. multocida*) and the mycotoxin FB₁, while in **Experiment 3** the lesions due to the interaction of *M. hyopneumoniae* and the FB₁ mycotoxin were studied.

The experimental design used in the three experiments is illustrated in Tables 2–4.

Table 2: Arrangement of treatment groups in Experiment 1

Room	Group	Number of piglets	Bb infection (0.5 ml/IT)
I	A	10	–
II	B	20	on day 4*

Bb = *Bordetella bronchiseptica*

IT = intratracheal infection through an endotracheal tube

* the piglets arrived on day 0, the indicated number represents the day of experiment

Table 3: Arrangement of treatment groups in Experiments 2

Room	Group	Number of piglets	Bb infection (0.5 ml/IT)	Pm infection (0.5 ml/IT)	FB ₁ treatment (10 mg/kg of feed)
I	A	7	–	–	–
	B	7	–	–	from day 16 to day 39 (to the end of the experiment)*
II	C	7	on day 4*	on day 16*	–
	D	7	on day 4*	on day 16*	from day 16 to day 39 (to the end of the experiment)*

Bb = *Bordetella bronchiseptica*, Pm = *Pasteurella multocida* serotype D

IT = intratracheal infection through an endotracheal tube

* the piglets arrived on day 0, the indicated numbers represent the days of experiment

Table 4: Arrangement of treatment groups in Experiment 3

Room	Group	Number of piglets	FB ₁ treatment (20 mg/kg of feed)	Mh infection (0.5 ml/IT)
I	A	7	–	–
	B	7	from day 16 to day 58 (to the end of the experiment)*	–
II	C	7	–	on day 30*
	D	7	from day 16 to day 58 (to the end of the experiment)*	on day 30*

Mh = *Mycoplasma hyopneumoniae*

IT = intratracheal infection through an endotracheal tube

* the piglets arrived on day 0, the indicated numbers represent the days of experiment

Experiments 1 and 2 lasted 39 days whereas Experiment 3 lasted 58 days, which duration was chosen on the basis of data available in the literature on the selected pathogens.

4.3. Methods applied during the study

Of the modern imaging modalities, we used CT for monitoring the changes induced in the lungs. This was complemented by bacteriological and serological tests as well as sphingolipid profile tests of the blood during the experiment and by gross and histopathological examinations carried out at the end of the experiment (Table 5).

Table 5: Examinations/tests performed and the time of their performance in the three experiments

Experiment	Test performed	Time-points of the tests performed*
Experiment 1	clinical signs, body temperature	daily during the experiment
	weighing CT examination bacteriological examination serological examination	on days 4, 16, 25 and 39
	gross and histopathological examinations	on day 39
Experiment 2	clinical signs, body temperature	daily during the experiment
	weighing CT examination bacteriological examination serological test	on days 4, 16, 25 and 39
	sphingolipid profile test	on days 25 and 39
	gross and histopathological examinations	on day 39
Experiment 3	clinical signs, body temperature	daily during the experiment
	weighing CT examination bacteriological examination serological test sphingolipid profile test	on days 30, 44 and 58
	sphingolipid profile test	on day 58
	gross and histopathological examinations	on day 58

* the piglets arrived on day 0, the indicated numbers represent the days of experiment

The CT examinations were carried out at the Institute of Diagnostic and Oncoradiology of Kaposvár University. For sedation and premedication of the pigs, combinations of the following active ingredients were used: azaperone (Stresnil[®] Janssen, Beerse, Belgium, 4 mg/bwkg im.), ketamine (CP-Ketamin[®] 10%, CP-Pharma, Burgdorf, Germany, 10 mg/bwkg im.), xylazine (CP-Xylazine[®] 2%, CP Pharma, Burgdorf, Germany, 1 mg/bwkg, im.), atropine (Atropinum sulfuricum[®] 0.1%, EGIS, Budapest, Hungary, 0.04 mg/bwkg, im.).

After premedication, a balloon-type endotracheal tube was inserted into the pigs' trachea, then general anaesthesia was induced by the inhalation of isoflurane (Forane[®], Abbott Laboratories, Abbott Park, Illinois, USA, in a mixture with 2 v/v % of oxygen). Subsequently the animals were placed into the CT scanner in supine position on a special supporting structure. Artificial breath-holding was applied during the thoracic scan by closing the valve on the expiration tube. Scans of the entire volume of the lungs were made with a SIEMENS Somatom Emotion 6 multislice CT scanner (Siemens, Erlangen, Germany) with a tube voltage of 130 kV, a dose of 100 mAs, and a field of view of 200 mm. From the collected data cross-sectional images of slices 2 and 5 mm thick were reconstructed, with full overlapping. The images of 2 mm thick slices were used for diagnostic, i.e. quality, evaluation, while from the images obtained with 5 mm thick slice thickness we collected quantitative data by the use of the Medical Image Processing software (MIP), version 1.0 (2006). On the cross-sectional images, the region of lung to be visualised was selected and demarcated by sample region (ROI = Region of Interest) with the help of a Wacom Bamboo MTE-450 digital table (Wacom Europe GmbH, Krefeld, Germany). The ray absorption units of pixels (HU, Hounsfield Units) from these regions of interest were added up, and their calculated mean values (mean density) formed the basis of quantitative analysis. The lung images archived in DICOM (.dcm) format were opened with the Medical Image Processing version 1.0 (MIP) programme. To facilitate easier discrimination of the lungs from the contiguous tissues, windowing from -700 to 300 was used during the demarcation of the regions of interest. With the help of the programme, the area selected for evaluation was framed, at an identical distance from the border of the lung tissue (Fig. 5).

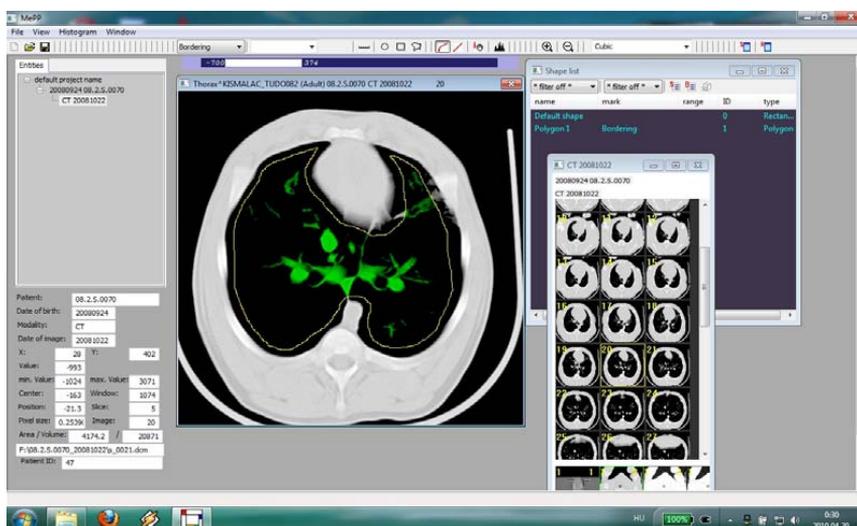


Figure 5: The interface of the MIP programme, showing the lung area selected for evaluation

Of the tools tried out for area selection and demarcation, the Wacom Bamboo MTE-450 digital table (Fig. 6) proved to be the most suitable. Its use increased the accuracy of, and markedly shortened the time needed for, area selection and demarcation.



Figure 6: The Wacom Bamboo MTE-450 digital table used for lung area selection and demarcation

During processing, particular attention must be paid to the borders of affected areas with the contiguous tissues, as the programme calculates the number of pixels belonging to the given HU (Hounsfield Unit) value from the demarcated areas, and a possible erroneous framing would distort the results obtained. From the framed areas of images in the same test series, the pixel frequencies belonging to the different HU values were saved in a frequency file of comma-separated values (csv) format, using a contraction of 10, in an HU range between minus 1000 and plus 200. The frequency tables of 'csv' format are directly readable by a Microsoft® Office Excel spreadsheet programme, which was used for the further processing of data.

4.4. Statistical analysis

The statistical model included treatment, age, and their effect on clinical, CT, and histopathologic signs, which were studied by using multi-way analysis of variance (ANOVA). The significance of differences was tested by Tukey's and least significant difference (LSD) *post hoc* tests. Because a significant age \times treatment interaction occurred ($P < 0.05$), data were further subjected to two types of statistical analyses: within the same age (among the 4 treatments) and within the same treatment (between ages). Data were analysed by using the GLM procedure of SAS (SAS Institute, Cary, NC).

4.5. Approval of the experiments

The series of experiments involving experimental infection of pigs, the feeding of a diet contaminated with FB₁ toxin and the performance of CT studies was approved by the Food Chain Safety and Animal Health Directorate of the Somogy County Government Office (legal predecessor: Animal Health and Food Control Station of Somogy County); approval number: 438/2/SOM/2005.

5.1. CHAPTER

Non Invasive (CT) Investigation of the Lung in *Bordetella bronchiseptica* Infected Pigs

Non Invasive (CT) Investigation of the Lung in *Bordetella bronchiseptica* Infected Pigs

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Summary

Bordetella bronchiseptica produced pneumonia was studied in young piglets. At the beginning of the experiment, 30 artificially reared 3-day-old piglets were divided into two groups: group A – uninfected piglets, control group (n=10) and group B – piglets infected with *B. bronchiseptica*, experimental group (n=20). The *B. bronchiseptica* infection (10⁶ CFU/ml) was performed on day 4. In Group B, clinical signs including mild serous nasal discharge, sneezing, panting, and hoarseness appeared from day 4. Computed tomography (CT) performed on day 16 demonstrated lung lesions attributable to colonisation by *B. bronchiseptica* in the infected pigs. The gross pathological findings confirmed the results obtained by CT.

Key words

Bordetella bronchiseptica, Porcine respiratory disease complex, Computed tomography

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Aim

Respiratory disease is one of the most important health concerns for modern swine production. The term porcine respiratory disease complex (PRDC) refers to a condition in which an interaction between various pathogens and inappropriate environmental conditions lead to severe respiratory disorders. Disease entities occurring in the simultaneous presence of multiple pathogens coupled with environmental predisposing factors are common in the practice and they have enormous importance for the profitability of production.

PRDC primary pathogens can be viruses or bacteria, while the secondary pathogens are mostly bacteria (Brockmeier et al., 2002a). *B. bronchiseptica* is frequently isolated from respiratory conditions produced by multiple aetiological factors. Its dermonecrotic toxin (DNT) has a fundamental role in producing respiratory disease in swine (Brockmeier et al., 2002b). Previous studies suggest that the concurrent presence of *B. bronchiseptica* and other respiratory pathogens develops more severe disease than that produced by infection with *B. bronchiseptica* alone (Brockmeier et al., 2000; Brockmeier, 2004; Brockmeier et al., 2008). *B. bronchiseptica* and toxigenic *P. multocida* are known to work together in producing the progressive form of porcine atrophic rhinitis (Chanter et al., 1989). *B. bronchiseptica* has also been demonstrated to be able to produce pneumonia in young piglets (Underdahl et al., 1982).

In the present study we examined the *B. bronchiseptica* produced pneumonia in young piglets. Computed tomography (CT) was applied to follow up the pathological events in the lung.

Material and methods

Thirty 3-day-old pigs were used in the study. The piglets included in the experiment originated from a herd of high health status, in which the incidence of respiratory diseases was negligible. The sows were free from toxigenic *B. bronchiseptica*. On the 3rd day of life, a total of 30 female piglets were selected, and transported to the experimental animal facility (day 0 of the experiment). After an early weaning, the piglets were artificially reared with milk-replacer until day 16 and then with solid feed until the termination of the experiment (day 39). On the day of their arrival, the piglets were placed into battery cages in two rooms. Two groups were assigned separately in two rooms: group A – uninfected piglets, control group (n=10) and group B – piglets infected with *B. bronchiseptica*, experimental group (n=20). Air temperature was adjusted to 27°C. The cages and the rooms were cleaned twice a day, and the piglet-rearing equipment was cleaned every second day. Animal tenders wore protective clothing, and disinfected their hands and feet with the aqueous solution of Virkon S[®] (Antec, Novo Mesto, Croatia) when entering the rooms. Up to day 16, the piglets were fed a milk replacer diet consisting of skim milk powder, vegetable fats and whey powder, containing 23% crude protein, 23% ether extract and 1.6% lysine (Salvana Ferkel Ammen Milch[®], Salvana Tiernahrung, Sparrieshoop, Germany) from 'Mambo' automatic feeder (Sloten, Deventer, The Netherlands). From day 7, a dry coarse meal containing 16 MJ/kg metabolizable energy, 18.5% crude protein, 9% ether extract and 1.65% lysine (Salvana Pre-meal[®], Salvana Tiernahrung, Sparrieshoop, Germany) was also

given to the piglets *ad libitum*, and then from day 16 up to the end of the experiment (day 39) only this latter diet was available to them. Drinking water was provided from nipple drinkers, and initially this was complemented with water offered from plastics drinking bowls of free water surface.

Group B piglets were infected with *B. bronchiseptica* (strain KM22, dose: 10⁶ CFU/mL) on day 4. The bacterial suspensions were prepared as described previously (Magyar et al., 2002). A volume of 0.5 mL was inoculated through an endotracheal tube in all cases.

The clinical signs were recorded daily during the experiment. The piglets were weighed on days 4, 16, 25 and 39.

CT examinations were performed on days 4, 16, 25 and 39 to detect lesions in the lung. Combinations of the following active ingredients were used for premedication: azaperone (Stresnil[®], Janssen, Beerse, Belgium, 4 mg/bwkg, i.m.), ketamine (CP-Ketamin 10%[®], CP-Pharma, Burgdorf, Germany, 10 mg/bwkg i.m.), xylazine (CP-Xylazine 2%[®], CP Pharma, Burgdorf, Germany, 1 mg/bwkg, i.m.), atropine (Atropinum sulphuricum 0.1%[®], EGIS, Budapest, Hungary, 0.04 mg/bwkg, i.m.). After premedication, a balloon-type endotracheal tube was introduced into the trachea, and then anaesthesia was induced by the inhalation of isoflurane (Forane[®], Abbott, Illinois, USA) in a mixture with 2% (v/v) oxygen. The animals were placed in supine position on a special supporting structure. To make CT scans, artificial breath-holding was applied during the thoracic scan. CT scans of the entire volume of the lungs were made with a SIEMENS Somatom Emotion 6 multislice CT scanner (Siemens, Erlangen, Germany); tube voltage: 130 kV, dose 100 mAs, FoV 200 mm). From the collected data cross-sectional images of 2- and 5-mm slice thickness were reconstructed, with full overlapping. The images were analysed using the Medical Image Processing (2006) software.

At termination, the pigs were humanly killed and lung lesions were examined post mortem. Post mortem examinations were performed on day 39. For histopathological examination, samples were taken from lung areas showing pathological changes. Tissue samples were fixed in 4% formalin solution, embedded in paraffin, sectioned, and the sections were stained with haematoxylin and eosin (HE).

Results and discussion

Piglets of Group A did not show clinical signs during the experiment. In Group B clinical signs including a mild serous nasal discharge, sneezing, panting and hoarseness appeared from 4 days after *B. bronchiseptica* infection. There were no significant difference between the groups in the growth rate of piglets (P>0.05). No lesions were seen in Group A at any of the test dates (Table 1).

On day 16, 25 and 39 lung lesions were seen in 19 out of the 20 piglets of Group B, which were characterised by a mild to moderate density increase (around –600/–300 on the Hounsfield scale, HU), as compared to the normal density in the pneumatised parenchymal areas of the lung (which is around –700/–800 HU). This density increase was the result of an inflammatory process (exudate formation, cell proliferation) (Figure 1).

Table 1. Number of piglets showing pathological lung lesions based upon the CT scan and the gross pathological examination performed at the end of the experiment as related to the total number of animals in the group

Group	Day of the experiment				Necropsy
	Day 4	Day 16	Day 25	Day 39	
A	0/10	0/10	0/10	0/10	0/10
B	0/20	19/20	19/20	19/20	19/20

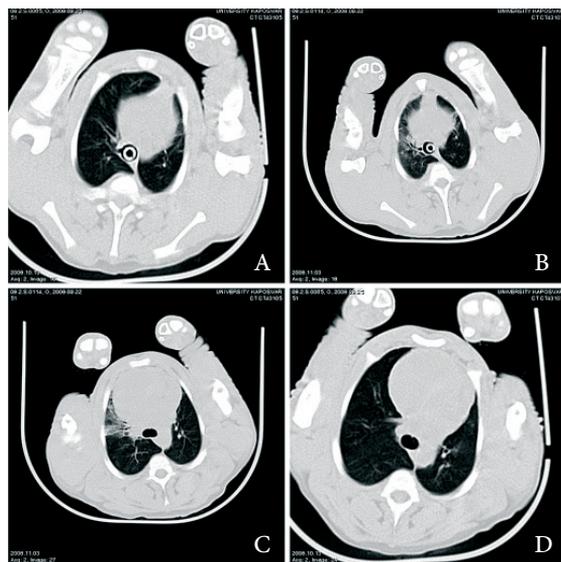


Figure 1. The affected areas in the lung of a *B. bronchiseptica* infected piglet with density increase on the CT scans (on day 16 and 39); A – anterior lobes on day 16, B – anterior lobes on day 39, C – intermediate lobes on day 16, D – intermediate lobes on day 39

In Group A none of the piglets had changes in the lung while the lungs of 19 out of the 20 surviving animals of Group B showed pathological lesions. These lesions were located mainly in the anterior and intermediate lobes and in the cranial third of the posterior lobe, and their size ranged from lesions involving a few lobules to changes extending to the entire lobe (Figure 2).

The lesions occurred mainly in the form of acute catarrhal pneumonia (Figure 2) with chronic catarrhal areas, hemorrhagia, pleuritis and fibrosis. Some animals developed combination of catarrhal and purulent or catarrhal and fibrinous pneumonia.

Conclusions

The *B. bronchiseptica* mono-infection was able to produce lung lesions in young pigs. Our results also indicate that CT can

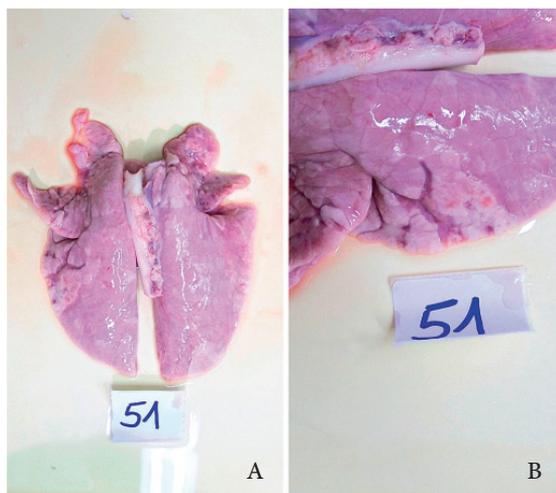


Figure 2. The location of the lung lesions of a *B. bronchiseptica* infected piglet (anterior, intermediate lobes and in the cranial third of the posterior lobe)

be applied for studying the pathological conditions in the lower respiratory tract of swine. Valuable information can be collected about the formation of the lesions over time as well as about the nature of the changes in the lung tissues.

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5.2. CHAPTER

Interaction of *Bordetella bronchiseptica*, *Pasteurella multocida* and fumonisin B1 in the porcine respiratory tract as studied by computed tomography

Interaction of *Bordetella bronchiseptica*, *Pasteurella multocida*, and fumonisin B1 in the porcine respiratory tract as studied by computed tomography

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Abstract

The interaction of *Bordetella bronchiseptica*, toxigenic *Pasteurella multocida* serotype D, and the mycotoxin fumonisin B₁ (FB₁) was studied. On day 0 of the experiment, 28 artificially reared 3-day-old piglets were divided into 4 groups ($n = 7$ each): a control group (A), a group fed FB₁ toxin (B), a group infected with the 2 pathogens (C), and a group infected with the 2 pathogens and fed FB₁ toxin (D). The *B. bronchiseptica* infection [with 10⁶ colony-forming units (CFU)/mL] was performed on day 4 and the *P. multocida* infection (with 10⁸ CFU/mL) on day 16. From day 16 a *Fusarium verticillioides* fungal culture (dietary FB₁ toxin content 10 mg/kg) was mixed into the feed of groups B and D. In groups C and D, clinical signs including mild serous nasal discharge, sneezing, panting, and hoarseness appeared from day 4, and then from day 16 some piglets had coughing and dyspnea as well. Computed tomography (CT) performed on day 16 demonstrated lung lesions attributable to colonization by *B. bronchiseptica* in the infected groups. By day 25 the number of piglets exhibiting lesions had increased, and the lesions appeared as well-circumscribed, focal changes characterized by a strong density increase in the affected areas of the lungs. The gross pathological findings confirmed the results obtained by CT. These results indicate that, when combined with dual infection by *B. bronchiseptica* and *P. multocida*, dietary exposure of pigs to FB₁ toxin raises the risk of pneumonia and increases the extent and severity of the pathological changes.

Résumé

L'interaction entre *Bordetella bronchiseptica*, *Pasteurella multocida* toxigène de sérotype D, et la mycotoxine fumonisine B₁ (FB₁) a été étudiée. Au jour 0 de l'expérience, 28 porcelets âgés de 3 j et élevés dans des conditions artificielles ont été séparés en 4 groupes de 7 porcelets : un groupe témoin (A), un groupe nourri avec la toxine FB₁ (B), un groupe infecté avec les 2 agents pathogènes (C), et un groupe infecté avec les 2 agents pathogènes et nourri avec la toxine FB₁ (D). L'infection avec *B. bronchiseptica* [10⁶ unités formatrices de colonies (UFC)/mL] a été effectuée au jour 4 et l'infection avec *P. multocida* (10⁸ UFC/mL) au jour 16. À partir du jour 16 une culture fongique de *Fusarium verticillioides* (contenu alimentaire en toxine FB₁ de 10 mg/kg) a été mélangée dans l'aliment des groupes B et D. Dans les groupes C et D, des signes cliniques incluant un léger écoulement nasal séreux, des éternuements, du halètement et une raucité sont apparus à partir du jour 4, et par la suite à partir du jour 16 certains porcelets présentaient de la toux ainsi que de la dyspnée. Une tomographie assistée par ordinateur (CT) effectuée au jour 16 a montré, dans les groupes infectés, des lésions pulmonaires attribuables à la colonisation par *B. bronchiseptica*. Au jour 25, le nombre de porcelets démontrant des lésions avait augmenté, et les lésions apparaissaient comme des zones focales de changements bien circonscrites, caractérisées par une forte augmentation de la densité dans les régions affectées du poumon. Les trouvailles pathologiques ont confirmé les résultats obtenus par CT. Ces résultats indiquent que, lorsque combinée à une infection mixte par *B. bronchiseptica* et *P. multocida*, l'exposition alimentaire des porcs à la toxine FB₁ augmente le risque de pneumonie et l'étendue et la sévérité des changements pathologiques.

(Traduit par Docteur Serge Messier)

Introduction

Porcine respiratory disease complex is a major health problem in modern pig production (1). Diseases occurring in the simultaneous presence of multiple pathogens coupled with environmental predisposing factors are common in this industry and have enormous importance for profitability. The primary pathogens can be viruses or bacteria; the secondary pathogens are mostly bacteria (1).

Bordetella bronchiseptica is frequently isolated from respiratory conditions produced by multiple etiologic factors. Its dermonecrotic toxin has a fundamental role in producing respiratory disease in swine (2). Research findings suggest that the concurrent presence of *B. bronchiseptica* and other respiratory pathogens results in more severe disease than infection with *B. bronchiseptica* alone (3–5). It is known that *B. bronchiseptica* and toxigenic *Pasteurella multocida* work together to produce the progressive form of porcine atrophic

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Table 1. Body weights in the piglet treatment groups (n = 7 each)

Day of experiment	Piglet group; mean weight ± standard deviation (kg)				P-value
	A	B	C	D	
4 ^a	2.14 ± 0.35	2.11 ± 0.33	1.95 ± 0.20	2.10 ± 0.15	0.561
16 ^b	3.08 ± 0.46	2.94 ± 0.50	2.79 ± 0.33	2.77 ± 0.27	0.442
25	5.13 ± 0.71	4.80 ± 0.92	4.64 ± 0.62	4.41 ± 0.89	0.443
39	9.08 ± 1.09	8.95 ± 1.56	8.94 ± 1.07	8.57 ± 2.67	0.959

A — control group; B — group fed fumonisin B₁ (FB₁); C — group infected with *Bordetella bronchiseptica* and *Pasteurella multocida* serotype D; D — group infected with *B. bronchiseptica* and *P. multocida* serotype D and fed FB₁.

^a Day of infection with *B. bronchiseptica*.

^b Day of infection with *P. multocida* and start of feeding with FB₁.

rhinitis (6), and *B. bronchiseptica* has been demonstrated to produce pneumonia in young piglets (7).

One of the pathogens most frequently isolated from the lungs is *P. multocida* (8). Capsular serotype A is believed to be dominant among the pulmonary isolates of *P. multocida* (9,10); however, some investigators have found no difference in the frequency of the 2 serotypes (A and D) isolated from the lungs (11). Studies on diseases caused by *P. multocida* in combination with other respiratory pathogens have demonstrated that mixed infections produce a more severe disease course (12–15).

Among the predisposing factors of environmental origin, mycotoxins occurring in feeds and exerting a harmful effect on the health of animals may play an important role. Fumonisin (FB₁, FB₂, FB₃, and FB₄), produced by *Fusarium verticillioides* (16), may be present worldwide, mainly in maize in considerable amounts (17,18). Short-term exposure of pigs to FB₁ toxin causes pulmonary edema; long-term exposure results in pulmonary fibrosis (19). Clinical signs develop only after exposure to high doses (more than 100 to 300 mg/kg of feed or 15 mg/kg body weight) of the toxin (20,21).

In this experiment the interactions of *B. bronchiseptica*, toxigenic *P. multocida*, and FB₁ toxin were studied to determine whether the toxin can influence the type or severity of pulmonary diseases of bacterial origin.

Materials and methods

Experimental animals and housing

The piglets included in the experiment originated from a herd of high health status in which the incidence of respiratory diseases was negligible. The sows were free from toxigenic *P. multocida*, and the prevalence of *B. bronchiseptica* infection was very low. On the 3rd day of life, 28 female piglets were selected and transported to the experimental animal facility.

On the day of their arrival (day 0 of the experiment) the piglets were placed in battery cages in 2 rooms. On day 16 the 14 piglets in each room were divided into 2 groups; thus there were 2 groups of 7 piglets each in both rooms. Group A, serving as controls, and group B, piglets to be fed FB₁ toxin, were housed in 1 room. Piglets to be experimentally infected with *B. bronchiseptica* and *P. multocida* (group C) as well as those to be infected with *B. bronchiseptica* and *P. multocida* and fed FB₁ toxin (group D) were kept in the 2nd room.

Air temperature was adjusted to 27°C. The cages and the rooms were cleaned twice a day, and the piglet-rearing equipment was cleaned every 2nd day. Animal tenders entering the rooms wore protective clothing and disinfected their hands and feet with an aqueous solution of Virkon S (Antec, Novo Mesto, Croatia) when entering the rooms.

Feeding of the animals

Up to day 16 the piglets were fed a milk replacer diet consisting of skim milk powder, vegetable fats, and whey powder that contained 23% crude protein, 23% ether extract, and 1.6% lysine (Salvana Ferkel Ammen Milch; Salvana Tiernahrung, Sparrieshoop, Germany) from a Mambo automatic feeder (Sloten, Deventer, the Netherlands).

From day 7 a dry coarse meal containing 16 MJ/kg of metabolizable energy, 18.5% crude protein, 9% ether extract, and 1.65% lysine (Salvana Pre-meal; Salvana Tiernahrung) was also given to the piglets ad libitum, and then from day 16 to the end of the experiment only the dry coarse meal was available to them.

Drinking water was provided from nipple drinkers, and initially this was complemented with water offered from plastic drinking bowls.

Experimental infection

Groups C and D were infected with *B. bronchiseptica* [strain KM22, 10⁶ colony-forming units (CFU)/mL] on day 4 and with toxigenic *P. multocida* serotype D (strain LFB-3, 10⁸ CFU/mL) on day 16. The bacterial suspensions were prepared as described previously (22). A volume of 0.5 mL was inoculated through an endotracheal tube in all cases.

Mycotoxin treatment

From day 16 until the end of the experiment (day 39) groups B and D were fed a diet into which an *F. verticillioides* fungal culture (23) containing 3691 mg/kg of FB₁ toxin was added in an amount to give a dietary FB₁ concentration of 10 mg/kg of feed. This diet and those fed to groups A and C were checked for mycotoxin content (17) and found not to contain detectable amounts of other mycotoxins (T-2, zearalenone, DON, and OTA).

Studies

Clinical signs were recorded daily during the experiment. The piglets were weighed on days 4, 16, 25, and 39.

Table II. Numbers of piglets with pathological lung lesions detected by computed tomography and by gross examination at necropsy^a

Piglet group	Day of experiment				Necropsy
	4	16	25	39	
A	0/7	0/7	0/7	0/7	0/7
B	0/7	0/7	0/7	0/7	0/7
C	0/7	3/7	3/6	3/6	3/6
			(4/7) ^b	(4/7) ^b	(4/7) ^b
D	0/7	5/7	5/6	4/5	4/5
			(6/7) ^b	(6/7) ^b	(6/7) ^b

^a Three piglets died during the experiment: 1 piglet on day 17 in group C and 2 piglets on days 24 and 34, respectively in group D. The 3 piglets had severe dyspnea and the characteristic signs of hypoxia.

^b Number of piglets with lung lesions/total number of piglets at the start of the experiment.

Sphingolipid profile test

On 2 occasions (on days 25 and 39) the free sphinganine to sphingosine ratio, the most sensitive biomarker of fumonisin toxicosis (24), was determined in the blood by a method described previously (25).

Computed tomography (CT)

On days 4, 16, 25, and 39 CT was used to detect lesions in the lung. Combinations of the following active ingredients, administered intramuscularly, were used for premedication: azaperone (Stresnil; Janssen Pharmaceutica, Beerse, Belgium), 4 mg/kg of body weight (BW); ketamine (CP-Ketamin 10%; CP-Pharma, Burgdorf, Germany), 10 mg/kg BW; xylazine (CP-Xylazine 2%; CP Pharma), 1 mg/kg BW; and atropine (Atropinum sulphuricum 0.1%; EGIS, Budapest, Hungary), 0.04 mg/kg BW.

After premedication a balloon-type endotracheal tube was introduced into the trachea, and then anesthesia was induced through inhalation of isoflurane (Forane; Abbott Laboratories, Abbott Park, Illinois, USA) in a mixture with 2% (v/v) oxygen. The animal was placed supine on a special supporting structure. Artificial breath-holding was applied during the thoracic scan.

Scans of the entire volume of the lungs were made with a Somatom Emotion 6 multislice CT scanner (Siemens, Erlangen, Germany) with a tube voltage of 130 kV, a dose of 100 mAs, and a field of view of 200 mm. From the collected data cross-sectional images of slices 2 and 5 mm thick were reconstructed, with full overlapping. The images were analyzed with the use of Medical Image Processing software (version 1.0, Ferenc Závoda, Kaposvár, Hungary).

Gross and histopathological examination

Postmortem examinations were performed on day 39. Macroscopic examination of the lungs was performed as described for routine slaughter check (26). Results were expressed as the percentage of lung area affected. For histopathological examination, samples were taken from lung areas showing pathological changes. Tissue samples were fixed in 4% formalin solution, embedded in paraffin, and sectioned; the sections were stained with hematoxylin and eosin.

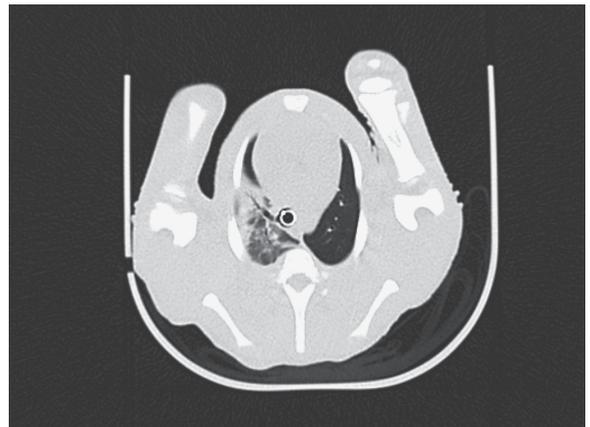


Figure 1. Lung lesions at the level of the 4th thoracic vertebrae on day 16 as shown by computed tomography (CT) of the lung of a piglet in group D infected with *Bordetella bronchiseptica* and *Pasteurella multocida* serotype D and fed fumonisin B₁ (FB₁).

Statistical evaluation

Differences between the groups were studied by 1-way analysis of variance (ANOVA) and Tukey's post hoc test with use of the SAS 9.1 program (SAS System for Windows, release 9.1; SAS Institute, Cary, North Carolina, USA). The level of statistical significance was set at $P \leq 0.05$.

Authorization

The experimental infection and CT examinations applied in this study were authorized by the Food Chain Safety and Animal Health Directorate of the Somogy County Agricultural Office (permission 438/2/SOM/2005).

Results

Clinical signs and weight

Groups A and B did not show clinical signs during the experiment. In groups C and D, clinical signs including mild serous nasal discharge, sneezing, panting, and hoarseness appeared from day 4 after *B. bronchiseptica* infection, and then from day 16 (infection with *P. multocida* and start of FB₁ toxin consumption) some piglets had coughing and dyspnea as well. The number of pigs with clinical signs was the same as the number with lung lesions: 4 out of 7 in group C and 6 out of 7 in group D.

Three piglets died during the experiment: 1 piglet on day 17 in group C and 2 piglets on days 24 and 34, respectively in group D. The 3 piglets had severe dyspnea and the characteristic signs of hypoxia.

No significant differences were found between the groups in the weight gain of the piglets (Table I). However, the growth rate of those in group D was somewhat inferior to that of the animals in the other 3 groups ($P > 0.05$). The piglets in the mycotoxin-fed groups (B and D) showed a pronounced heterogeneity of body weight on day 39, as indicated by the rather high standard deviations.

Blood sphingolipid profile

On day 39 the free sphinganine to sphingosine ratio in the blood was elevated ($P < 0.05$) in groups B and D (0.65 and 0.47, respectively) as compared with the groups not fed FB_1 toxin (0.22 and 0.27, respectively), indicating the effect of the toxin at the cellular level.

Evaluation of the CT scans

No lung lesions were seen in groups A and B on any of the test dates. Table II presents the numbers of piglets in groups C and D with lesions on the various test dates.

On day 16 lung lesions (Figure 1) were seen in 3 piglets in group C and 5 piglets in group D. The lesions were characterized by a mild or moderate density increase [about $-600/-300$ Hounsfield units (HU)], as compared with the normal density in the pneumatized parenchymal areas of the lung (about $-700/-800$ HU). This density increase was the result of an inflammatory process (exudate formation and cell proliferation).

By day 25 the number of piglets showing well-circumscribed, focal lung lesions had increased. As a result of chronic inflammation, necrotic processes, and connective tissue formation, the affected lung areas showed foci of very high density (0 to 150 HU) surrounded by a zone of low or medium density (Figure 2).

Gross and histopathological findings

In groups A and B none of the piglets had changes in the lung. In contrast, the lungs of 3 of the 6 surviving animals in group C and 4 of the 5 surviving animals in group D showed pathological lesions (Table II). The average percentage of affected lung area was $8.9\% \pm 16.0\%$ (standard deviation) in group C and $16.9\% \pm 22.3\%$ in group D ($P = 0.087$).

The lesions were located mainly in the anterior and intermediate lobes and in the cranial third of the posterior lobe. Involvement ranged from a few lobules (Figure 3A) to the entire lobe (Figure 3B). The lesions occurred mainly in the form seen in chronic catarrhal pneumonia, with necrotic foci demarcated by a fibrous capsule. Areas showing acute catarrhal changes were also observed around the chronic lesions. In 6 piglets a chronic adhesive pleuritis had also developed. The piglets that died during the experiment typically had acute or subacute serous-hemorrhagic catarrhal pneumonia.

The histopathological changes observed in group C included an acute serous-hemorrhagic catarrhal pneumonia in the piglet that died on day 17 (with infiltration of lymphocytes, histiocytes, and neutrophil granulocytes in the lumen of the alveoli), which was seen also in the piglets undergoing necropsy at the end of the experiment. In addition, alveolar emphysema, focal atelectasis, catarrhal infiltration, fibrotic encapsulation, necrosis, and subacute pleuritis occurred (Figure 4). In group D the changes seen in group C were accompanied by mild or moderate alveolar and interstitial edema in some animals (Figure 5).

Discussion

The negative impact of respiratory pathogens (including *B. bronchiseptica* and *P. multocida*) on the weight gain of piglets has been demonstrated by several studies (3,7,27). Tóth et al (28) found that

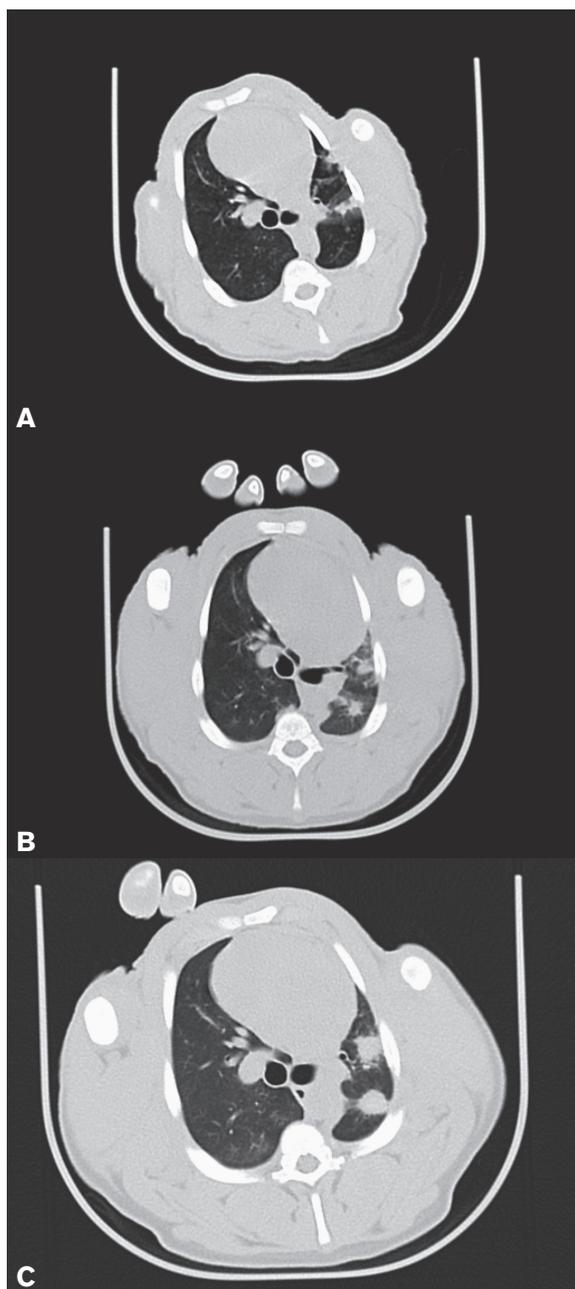


Figure 2. Demonstration of the progressively focal character of the lung lesions in serial CT scans of a piglet in group C on days 16 (A), 25 (B), and 39 (C) at the level of the 6th thoracic vertebra.

FB_1 toxin did not affect feed intake and body weight gain even at a dose of 40 mg/kg of feed, despite the fact that such levels of FB_1 cause rather severe but not clinically manifest pulmonary edema. On the other hand, Halloy et al (29) detected a significant depression in the body weight gain of toxin-fed piglets when the effects exerted



Figure 3. Diffuse inflammation extending to a lung lobule in the pulmonary parenchyma (A) and chronic inflammation of the pulmonary parenchyma extending to most of the left anterior, intermediate, and posterior lobes, accompanied by adhesive pleuritis (B), in piglets in group D.

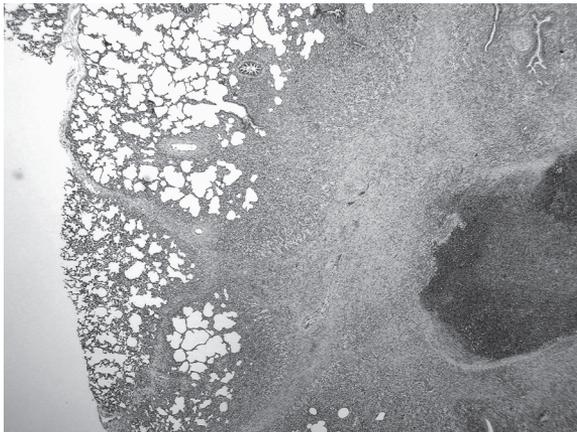


Figure 4. Inflammatory and necrotic foci surrounded by a fibrous capsule in the pulmonary parenchyma of a piglet in group C. Hematoxylin and eosin (H&E); original magnification $\times 20$.

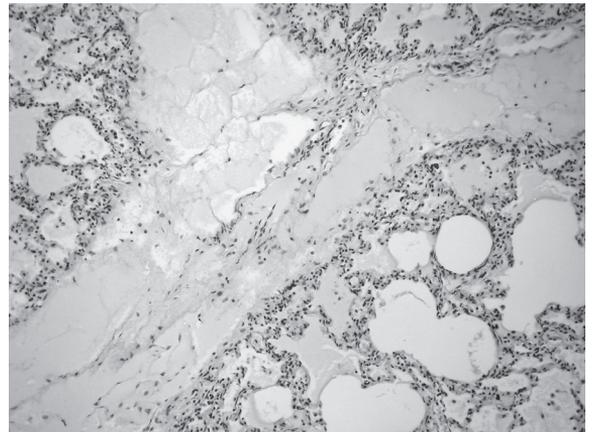


Figure 5. Alveolar and interstitial edema in the lung of a piglet in group D. H&E; original magnification $\times 100$.

by the combination of FB_1 toxin and *P. multocida* were studied in experimentally infected piglets. The role played by FB_1 toxin in dual infection with *B. bronchiseptica* and *P. multocida* and the interactions of the toxin and these pathogens had not yet been studied. In the present experiment there was no significant difference found in

the average body weight of the groups, although the infected and FB_1 -treated group D piglets had the lowest growth rate among the groups.

The results of this study support the earlier finding that in young piglets *B. bronchiseptica* infection can cause lung lesions (7,30).

Infection with *P. multocida* and the dietary intake of FB₁ toxin starting 12 d after *B. bronchiseptica* infection increased the incidence of clinical signs, indicating an interaction between the bacterial infections and the mycotoxin.

The progressive nature of the pneumonia was confirmed with serial CT examination. By day 25, 71% of the piglets in the infected groups (57% of those in group C and 86% of those in group D) had pathological lung lesions that were increasing in size and becoming progressively focal. By the end of the experiment (day 39) the focal pneumonic nature had become more pronounced, whereas its severity was similar to that found on day 25.

At necropsy the incidence of lung lesions was the highest and their extent the most pronounced in the piglets of group D. This finding is in accord with the observation of Halloy et al (29) that *P. multocida* produces more severe and more extensive pneumonic lesions in piglets also exposed to fumonisin B₁ toxin.

From the results of this experiment it can be concluded that, when coupled with dual infection by *B. bronchiseptica* and *P. multocida*, dietary exposure to the mycotoxin FB₁ above the advised level of 5 mg/kg of feed (31) raises the risk of pneumonia and increases the extent and severity of the pathological changes produced.

Computed tomography is potentially suitable for the early detection of pneumonia and for monitoring its course and could thus provide useful information for the study of other respiratory conditions. We are currently designing a method to quantify the extent of lung lesions detected on CT scans that may improve the applicability of this technique.

Acknowledgement

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5.3. CHAPTER

Use of Computed Tomography and Histopathologic Review for Lung Lesions Produced by the Interaction Between *Mycoplasma hyopneumoniae* and Fumonisin Mycotoxins in Pigs

Use of Computed Tomography and Histopathologic Review for Lung Lesions Produced by the Interaction Between *Mycoplasma hyopneumoniae* and Fumonisin Mycotoxins in Pigs

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Abstract

Mycoplasma hyopneumoniae has a primary role in the porcine respiratory disease complex (PRDC). The objective of this study was to determine whether fumonisin mycotoxins influence the character and/or the severity of pathological processes induced in the lungs of pigs by *Mycoplasma hyopneumoniae*. Four groups of pigs ($n = 7/\text{group}$) were used, one fed 20 ppm fumonisin B₁ (FB₁) from 16 days of age (group F), one only infected with *M. hyopneumoniae* on study day 30 (group M), and a group fed FB₁ and infected with *M. hyopneumoniae* (group MF), along with an untreated control group (group C). Computed tomography (CT) scans of infected pigs (M and MF) on study day 44 demonstrated lesions extending to the cranial and middle or in the cranial third of the caudal lobe of the lungs. The CT images obtained on study day 58 showed similar but milder lesions in 5 animals from group M, whereas lungs from 2 pigs in group MF appeared progressively worse. The evolution of average pulmonary density calculated from combined pixel frequency values, as measured by quantitative CT, was significantly influenced by the treatment and the age of the animals. The most characteristic histopathologic lesion in FB₁-treated pigs was pulmonary edema, whereas the pathomorphological changes in *Mycoplasma*-infected pigs were consistent with catarrhal bronchointerstitial pneumonia. FB₁ aggravated the progression of infection, as demonstrated by severe illness requiring euthanasia observed in 1 pig and evidence of progressive pathology in 2 pigs (group MF) between study days 44 and 58.

Keywords

computed tomography, fumonisin B₁, histopathology, *Mycoplasma hyopneumoniae*, pig

The porcine respiratory disease complex (PRDC) mainly affects growing and finishing pigs and reduces animal growth and profitability.^{3,29,37} It is caused by the combined effects of multiple pathogens and predisposing environmental factors. *Mycoplasma hyopneumoniae* was first isolated in the 1960s from pigs suffering from respiratory disease and later became known as enzootic pneumonia of pigs.^{13,19} Subsequently, *M. hyopneumoniae* was identified as one of the major causative factors in PRDC.

Among the predisposing environmental factors reported for PRDC, mycotoxins present in the diet may exert a deleterious effect on the health of pigs already infected with *M. hyopneumoniae*. Fumonisin, such as fumonisin B₁ (FB₁), fumonisin B₂ (FB₂), fumonisin B₃ (FB₃), and so on, are secondary metabolites of the mold species, *Fusarium verticillioides* (Sacc.) Nirenberg (previously called *Fusarium moniliforme* Sheldon),¹² which may occur in considerable amounts in various grains (particularly maize) all over the world.⁷ The mycotoxin FB₁ is considered responsible for inducing porcine pulmonary

edema¹⁵ and pulmonary fibrosis that develops in cases of chronic exposure.⁴¹ It may cause immunosuppression^{20,33} and has been associated with secondary infections, including intestinal colonization by pathogenic *Escherichia coli*,²² or respiratory pathogenic agents including *Pasteurella multocida*¹⁴ and porcine reproductive and respiratory syndrome (PRRS) virus.²⁵

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Few studies have addressed the combined effect of FB₁ and respiratory pathogens in pigs. In one experiment,²⁵ more severe lung alterations were observed in animals consuming FB₁ and subsequently inoculated with PRRS virus. Increased lesion severity was thought to be due to the immunosuppressive effect of the toxin, which allowed the virus to progress. The immunosuppressive effect of the toxin may be related to the accumulation of free sphingoid bases, which inhibits the proliferation of lymphocytes.³⁴ Others²² demonstrated that dietary exposure to FB₁ decreased resistance to *E. coli*, resulting in increased bacterial colonization of the small intestine. The effect of FB₁ on *P. multocida* infection was theorized to be related to the suppressed immune response and the reduced phagocytic ability of pulmonary macrophages.¹⁴ Increased susceptibility of pulmonary capillaries to FB₁ may also render the lungs more prone to infection.¹⁴ Several studies have also demonstrated that FB₁ mycotoxin has a proinflammatory effect.³⁴

Previous studies have shown that even low contamination levels of FB₁ (10 ppm) can decrease the immune response in pigs as expressed by the significant decrease in antibody titers against Morbus Aujeszky at days 21 and 35 postvaccination.³³ Another study found that feeding a diet containing 10 mg FB₁ per kilogram of feed, in combination with *Bordetella bronchiseptica* and toxigenic *Pasteurella multocida* serotype D infection, increased the incidence, extension, and severity of pulmonary lesions in pigs.²³

This study reviews the interaction of *M. hyopneumoniae*, recognized as one of the most important porcine respiratory pathogens, with fumonisin toxins in the lungs of pigs by both computed tomography (CT) and histopathologic examination. The objective was to determine whether FB₁ given at feed levels encountered in a production environment can influence the character and/or the severity of the pathological processes induced by *M. hyopneumoniae* in the lungs of pigs. The level of FB₁ contamination used in the present study (20 mg/kg feed) corresponded to the higher levels observed in naturally contaminated feed for pigs.^{31,32}

Materials and Methods

Experimental Animals and Their Management

Piglets were obtained from a Seghers hybrid herd that was free from brucellosis, leptospirosis, Aujeszky disease, and PRRS and in which the incidence of respiratory diseases was low. The sows delivering the piglets selected for the experiment ($n = 10$) were serologically negative for *M. hyopneumoniae*.

Piglets were randomly divided into 4 groups using the principle of equality, so that the average body weight of the groups was 2.35 ± 0.20 kg on day 3 of life. The 4 groups were split into 2 separate rooms on the day of arrival, which corresponded with day 0 of the study. The 2 uninfected groups, control animals (group C, $n = 7$) and pigs fed FB₁ toxin (group F, $n = 7$), were kept in one of the rooms, with the groups segregated in two separate elevated-level battery cages of identical size. The pigs infected with *M. hyopneumoniae* (group M,

$n = 7$) and those infected with *M. hyopneumoniae* and fed FB₁ toxin (group MF, $n = 7$) were housed in the second room and were similarly segregated in 2 battery cages. Both rooms were kept at air temperature (27°C) with the required air exchange ensured by exhaust fans. The battery cages, the drinking systems, and the rooms were cleaned twice a day, and the piglet-rearing installations were dismantled and washed every second day. All operators were required to wear protective clothing and perform foot and hand disinfection with an aqueous solution of Virkon S (KRKA d. d.; Antec International Ltd, Novo Mesto, Croatia) upon entry and exit from the rooms.

Feeding of the Experimental Animals

The piglets received a milk replacer consisting of skimmed milk powder, vegetable fats, and whey powder, containing 23% crude protein, 23% crude fat, and 1.6% lysine (Salvana Ferkel Ammen Milch; Salvana Tiernahrung GmbH, Klein-Offenseth Sparrieshoop, Germany), from an automatic feeder (Sloten B.V., Deventer, the Netherlands) until day 16 of the study. Beginning on day 7, piglets also received a dry meal ad libitum, containing 16 MJ/kg energy, 18.5% crude protein, 9% crude fat, and 1.65% lysine (Salvana Pre-meal; Salvana Tiernahrung GmbH). From day 16 until the end of the experiment (day 58), milk replacer was eliminated and only the dry meal made available.

Drinking water was available from nipple drinkers throughout the study. Between days 0 and 7, water was also provided from plastic drinking bowls.

Mycotoxin Treatment

F. verticillioides fungal culture containing 3691 mg/kg FB₁, 650 mg/kg FB₂, and 350 mg/kg FB₃ was produced by previously described methods.⁹ Beginning on study day 16, a defined quantity of the fungal culture was dried, ground, intimately homogenized, and step by step diluted and homogenized with the dry meal to give the required concentration of 20 mg/kg feed FB₁ (3.5 mg/kg FB₂ and 1.9 mg/kg FB₃). The toxin-containing diet was fed to groups F and MF until the end of the study (day 58), for a period totaling 42 days.

The mycotoxin contents of the fungal culture alone, the normal dry meal diet, and the artificially mycotoxin-contaminated dry diet were checked prior to feeding using a liquid chromatography–mass spectrometry (LC-MS) system (LC-MS 2020 Single Quadrupole Mass Spectrometer, LC-20 AD pumps with DGU-20A degasser, SIL-20ACHT autosampler, CTO-20-AC Column Owen, and CBM-20A Interface; SHIMADZU, Kyoto, Japan). The detection limits for FB₁, T-2, zearalenone, deoxynivalenol, ochratoxin A, and aflatoxin B₁ were 10, 3, 5, 3, 10, and 10 µg/kg, respectively. No other mycotoxins (including T-2, zearalenone, deoxynivalenol, ochratoxin A, aflatoxin B₁) were present in detectable quantities in the fumonisin fungal cultures or contaminated diets fed to groups F and MF. The basal diet was also free of fumonisin contamination.

Experimental Infection and Microbiological Investigations

On study day 30, pigs from 2 groups (M and MF) were infected with a virulent strain of *M. hyopneumoniae* obtained from a herd experiencing clinical disease. The pigs were inoculated intratracheally with a lung homogenate suspended in Friis medium¹¹ containing 3×10^5 color-changing units of *M. hyopneumoniae* in a volume of 3 ml. Control animals (group C) were treated similarly but with sterile Friis medium. Preliminary investigations revealed no pathological response in the lungs of pigs inoculated with the sterile medium. Microbiological investigations revealed no respiratory pathogens in the inoculums, in the lungs of the control and infected pigs at the end of the study, or in the single-group MF pig that died during the experiment, except the *M. hyopneumoniae* pathogen. *M. hyopneumoniae* was detected only in the experimentally infected pigs by polymerase chain reaction (PCR)² on bronchoalveolar lavage fluid collected at necropsy.

Samplings and Test Parameters

Clinical signs and body temperatures were recorded daily. Piglets were weighed and blood samples were taken on study days 30, 44, and 58.

Serology

Blood samples from infected and control pigs were tested for *M. hyopneumoniae* antibodies with the DAKO Mh ELISA (DAKO, Glostrup, Denmark).

Sphingolipid Profile Test

On study day 58, the free sphinganine to sphingosine ratio, known to be the most sensitive biomarker of fumonisin toxicosis,²¹ was determined using a previously described method.¹⁷

Computed Tomography Examination

CT examination to detect lesions in the lung was performed on study days 30, 44, and 58. Combinations of the following active ingredients were used for narcosis and premedication: azaperone (Stresnil [Janssen, Beerse, Belgium]; 4 mg/kg body weight [BW], intramuscular [IM]), ketamine (CP-Ketamin 10% [CP-Pharma, Burgdorf, Germany]; 10 mg/kg BW IM), xylazine (CP-Xylazine 2% [CP Pharma]; 1 mg/kg BW IM), and atropine (Atropinum sulphuricum 0.1% [EGIS, Budapest, Hungary]; 0.04 mg/kg BW IM). After premedication, a balloon-type endotracheal tube was inserted, and anesthesia was maintained with isoflurane inhalation (Forane; Abbott Laboratories, Abbott Park, IL) in a mixture with 2% (v/v) oxygen. Anesthetized pigs were placed into the CT machine in supine position on a special supporting structure. Artificial breath holding was applied during CT scanning of the lungs by occluding the expiratory valve. For the duration of the thoracic scans, intrapulmonary pressure was kept on a standard value (5 kPa).

CT scans of the entire lung region were taken with a SIEMENS Somatom Emotion 6 multislice CT scanner (Siemens, Erlangen, Germany) using the following parameters: tube voltage, 130 kV; dose, 100 mAs; and FoV [field of view], 200 mm. From the collected data, 2- and 5-mm-thick cross-sectional images were reconstructed, with full overlap. Scans of 2-mm slice thickness were used for diagnostic (ie, qualitative) evaluation, whereas 5-mm slice thickness scans collected quantitative data using the Medical Image Processing software (version 1.0, Ferenc Závoda, Kaposvár, Hungary). On cross-sectional images, the regions of interest (ROIs) of the imaged lung were marked out with the help of a Wacom Bamboo MTE-450 digital table (Wacom Europe GmbH, Krefeld, Germany). The x-ray absorption values (Hounsfield units, HU) of pixels from these ROIs were added, and their calculated average values (average density) formed the basis of quantitative analysis.

Gross Pathology and Histopathologic Examination

At the end of the study period (day 58), the pigs were humanely euthanized and then subjected to postmortem examination. Lung samples were taken for histologic examination and fixed in 10% neutral buffered formalin. Fixed tissues were embedded in paraffin wax, sectioned at 4 μ m, and stained with hematoxylin-eosin (HE). Periodic acid-Schiff (PAS) stain was also used for highlighting lipoprotein, glycoprotein, or mucoprotein substances in various tissues and cell components. Some materials were stained with Weigert iron hematoxylin for evaluation of the presence or absence of fibrin in edematous areas.

Statistical Analysis

The statistical model included treatment, age, and their effect on clinical, CT, and histopathologic signs, which were studied by using multiway analysis of variance (ANOVA). The significance of differences was tested by Tukey and least significant difference (LSD) post hoc tests. Because a significant age \times treatment interaction occurred ($P < .05$), data were further subjected to 2 types of statistical analyses: within the same age (among the 4 treatments) and within the same treatment (between ages). Data were analyzed by using the GLM procedure of SAS (SAS Institute, Cary, NC).

Animal Welfare Permission

The experimental infection and the CT examinations applied in this study were authorized by the Food Chain Safety and Animal Health Directorate of the Somogy County Agricultural Office, under permission number 23.1/02322/008/2008.

Results

Growth Rate and Clinical Signs

No significant differences in the body weights of the pigs between groups were observed (data not shown).

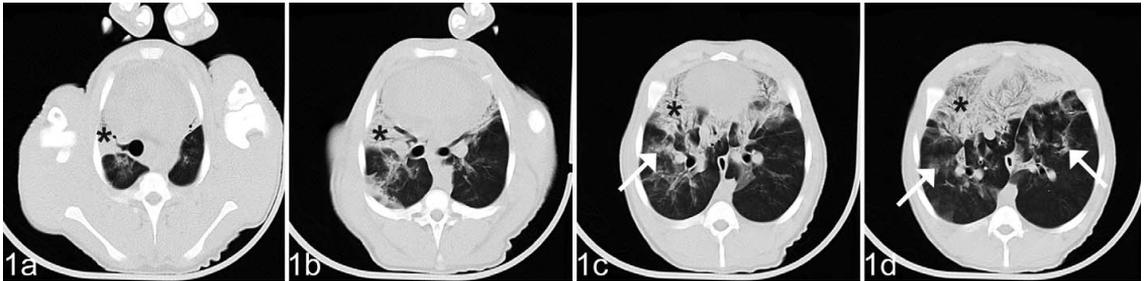


Figure 1. Lung; pig, group MF. Computed tomography images in different sectioning planes on study day 44 (day 14 after infection). Patchy ground glass opacification (arrows) and ventral consolidation (*).

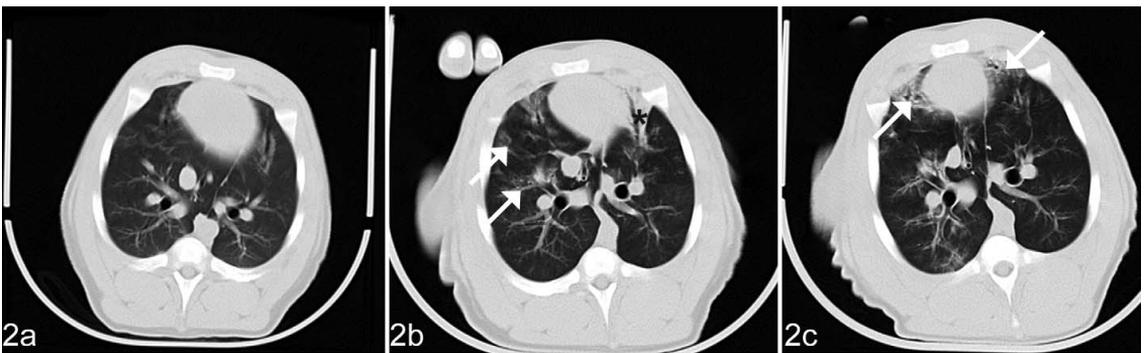


Figure 2. Lung; pig, group M. Computed tomography images showing time course of the development of lung lesions in a pig infected with *Mycoplasma hyopneumoniae* on study day 30 (a). Pigs treated only with *M. hyopneumoniae* showed gradual healing in the lungs observed by comparing the images taken on study day 44 (b) and study day 58 (c). Patchy ground glass opacification (arrows) and ventral consolidation (*).

During the study, 1 pig in group MF was euthanized on day 55 (39 days after the start of toxin feeding and 25 days after *M. hyopneumoniae* infection), due to clinical signs of progressive dyspnea and hypoxia (open-mouth breathing, cyanotic discoloration of the mucous membranes, and protruding body parts).

No clinical signs were observed in groups C and F during the study. Starting from day 7 after *M. hyopneumoniae* infection (study day 37), all infected animals (groups M and MF) exhibited mild and progressively increasing coughing or hoarseness, with some dyspnea. From day 4 postinfection (study day 34), infected animals (groups M and MF) had temporary febrile periods, whereas control and toxin-fed pigs (groups C and F) maintained normal physiological body temperatures (between 38.5°C and 39.5°C).

Serology

All pigs tested negative for *M. hyopneumoniae* antibodies in the DAKO Mh ELISA at the start of the study. All pigs infected with *M. hyopneumoniae* had seroconversion by 28 days postinfection.

Blood Sphingolipid Profile

On study day 58, the free sphinganine (SA) to sphingosine (SO) ratio (SA/SO), known to be a biomarker of FB₁ toxin, was statistically significantly elevated ($P < .05$) in groups F and MF (1.43 and 1.29, respectively) as compared with groups C and M not fed toxin (0.41 and 0.31, respectively), indicating that there was an effect exerted by the toxin at a cellular level.

CT Image Evaluation of Lung Lesions

The CT scans from study day 30 did not show appreciable lesions in the lungs of pigs in any of the groups. On study day 44, the CT scans of infected pigs (M and MF) showed lesions involving 1 or multiple neighboring lobules in the cranial and middle lung lobes, as well as in the cranial third of the caudal lung lobe (Fig. 1). Lesions consisted of patchy ground glass opacification, whereas more severe cases also had ventral consolidation.

CT images at the end of the study (day 58) showed similar lung lesions that were milder in 5 group M animals (Fig. 2) and 2 pigs in group MF but progressively aggravated in 2 other pigs in group MF (Fig. 3). Lesions indicative of pulmonary edema (groups F and MF) could not be identified on the CT images.

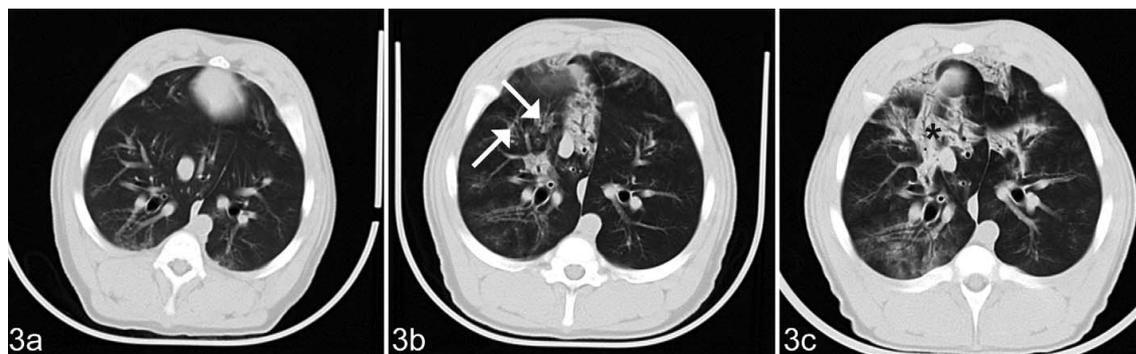


Figure 3. Lung; pig, group MF. Computed tomography images showing time course of the development of lung lesions in a pig infected with *Mycoplasma hyopneumoniae* and exposed to fumonisins on study day 30 (a). Pigs treated with both the bacteria and toxin showed progressive pulmonary disease by comparing the images taken on study days 44 (b) and 58 (c). Patchy ground glass opacification (arrows) and ventral consolidation (*).

Table 1. Result of the statistical analysis of density in the regions of interest of pulmonary parenchymal areas of the lungs on the Hounsfield scale (HU means \pm SD).

Group	Age			
	Day 30	Day 44	Day 58	Total
C	-638 \pm 48 ^A	-756 \pm 68 ^{aB}	-715 \pm 26 ^{aB}	-703 \pm 82 ^a
F	-648 \pm 60 ^A	-775 \pm 17 ^{aB}	-738 \pm 37 ^{aB}	-720 \pm 80 ^a
M	-634 \pm 49	-637 \pm 83 ^b	-656 \pm 36 ^b	-643 \pm 58 ^b
MF	-650 \pm 57	-606 \pm 115 ^b	-679 \pm 26 ^b	-644 \pm 84 ^b
Total	-643 \pm 51 ^A	-691 \pm 106 ^B	-697 \pm 373 ^B	

C, control; F, fed fumonisin; M, infected with *Mycoplasma hyopneumoniae*; MF, infected with *M. hyopneumoniae* and fed fumonisin. Different indices mean significant differences ($P < .05$) between ^{a,b}groups (within the same column) or ^{A,B}age (within the same row). $n = 7$ /group, except group MF on day 58, where $n = 6$ (1 animal in group MF was euthanized on day 55). Total mean and SD values are of all data in the same row (group) or column (age).

The evolution of average density calculated from combined pixel frequency values obtained for designated areas of the lungs (Table 1) was significantly influenced by the treatment and the age of animals at the time of the examination ($P = .024$). The average pulmonary density decreased between study days 30 and 44 ($P < .01$) but did not change between study days 44 and 58. The average density of the lungs of infected groups (M and MF) was 5% to 10% higher when compared with those of uninfected animals (groups C and F) ($P < .001$). The average pulmonary density values found in infected pigs (groups M and MF) on days 44 and 58 did not differ significantly from the values obtained on day 30. Treatment with FB₁ (groups F and MF) did not cause a significant difference in average pixel density in the case of both the uninfected (C and F) and the infected (M and MF) groups.

Evaluation of the Lung Lesions on the Basis of the Gross Pathological Findings

In group C, macroscopic lesions were not seen in the lungs of any of the pigs. In group F, there was mild interstitial edema in

the lungs of all pigs (Fig. 4). All animals in groups M and MF had developed subacute or acute catarrhal bronchointerstitial pneumonia. The lesions were located mainly in the cranial and middle lung lobes and in the cranial third of the caudal lung lobe and varied in severity by extending to a few lobules or involving the entire lobe. In group MF animals, mild interstitial edema involving the entire lung was seen in all animals in addition to the pneumonic changes (Fig. 5), and 2 pigs had also developed serofibrinous pleuritis and pericarditis. The pig that was euthanized on study day 55 (group MF) had acute to subacute catarrhal bronchointerstitial pneumonia and severe interstitial edema, along with serofibrinous pleuritis, pericarditis, and peritonitis.

Evaluation of the Lung Lesions on the Basis of the Histopathologic Findings

In the lungs of all examined pigs exposed to fumonisin (group F), there was thickening of interalveolar septa due to epithelial hyperplasia and accumulation of serofibrinous exudate or fibrin. There was also edema and accumulation of serous or serofibrinous exudates in the interlobular tissue of all group F pigs (Fig. 6), whereas pronounced perivascular edema and hyperemia of vessels were seen in 1 group F pig.

In the lungs of all pigs infected with *M. hyopneumoniae* (group M), there was thickening of interalveolar septa adjacent to bronchioles due to hyperplasia of type II alveolar epithelial cells and the presence of macrophages or lymphoid cells. Proliferation and hyperplasia of bronchial or bronchiolar epithelium, sometimes associated with cloudy swelling and eosinophilia, were often observed (Fig. 7). Accumulation of fibrin (fibrinous exudates) with prominence of leukocytes, macrophages, and desquamated epithelial cells was seen in the septa, alveolar spaces, and lumina of bronchi or bronchioles (Fig. 8) in all examined pigs.

Pathomorphological changes in lungs of infected and FB₁-fed pigs (group MF) corresponded to the morphologic

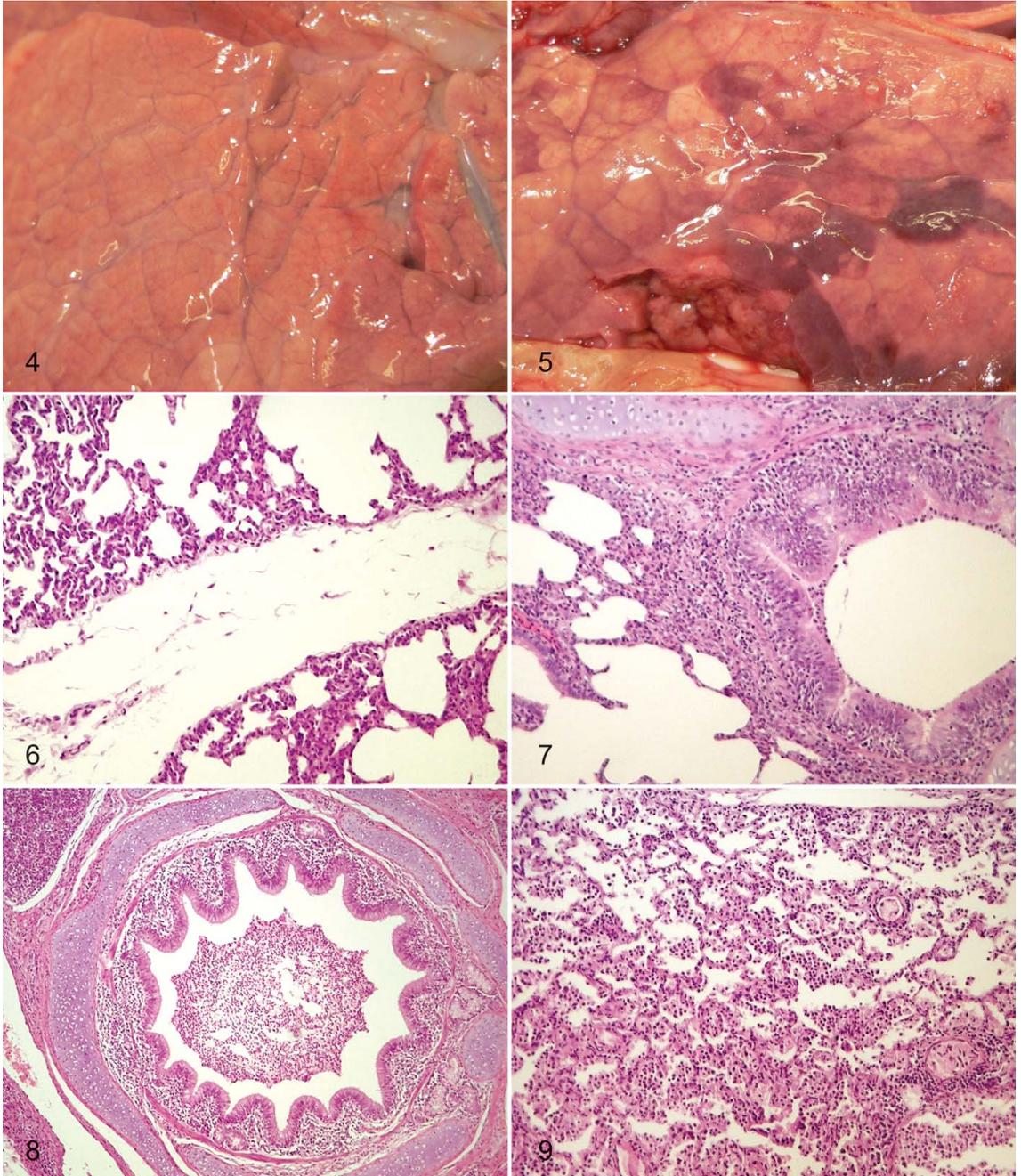


Figure 4. Lung; pig, group F. The interlobular septa are edematous on study day 58. There is no evidence of the lesions observed in the bacterially infected animal groups. **Figure 5.** Lung; pig, group MF. Pneumonic areas and slight thickening of the interlobular septa on study day 58. **Figure 6.** Lung; pig, group F. Accumulation of serous exudates and edema in the interstitium. Hematoxylin and eosin (HE). **Figure 7.** Lung; pig, group M. Thickening of interalveolar septa adjacent to bronchioles due to epithelial hyperplasia and macrophages or lymphoid cells in the septa. There is also proliferation of bronchial and/or bronchiolar epithelium. HE. **Figure 8.** Lung; pig, group M. Lymphocytic bronchitis accompanied

pattern of a catarrhal bronchiointerstitial pneumonia, with development of prominent peribronchial and peribronchiolar lymphocyte infiltration and a strong interstitial edema. The alveolar architecture was often obscured by thickened alveolar septa and atelectatic or exudate-filled lumina. There was thickening of interalveolar septa adjacent to bronchioles by hyperplasia of type II alveolar epithelial cells and the presence of macrophages or lymphoid cells. Proliferation and hyperplasia, along with cloudy swelling in some cells, of bronchial or bronchiolar epithelium were observed along with hyperplasia of the alveolar epithelium. Accumulation of fibrin with or without accumulation of leukocytes, macrophages, and desquamated epithelial cells in the interalveolar or interlobular septa, alveolar spaces, and bronchi or bronchioles was present in all group MF pigs (Fig. 9). Small pulmonary parenchymal hemorrhages were seen in some areas. Slight to moderate edema (serous exudates) (Fig. 9) or accumulation of fibrinous exudates was seen in the pleura and in the interlobular tissue in almost all group MF pigs.

No visible pathomorphological changes were seen in the lungs of the control pigs (group C), except slight hyperemia of some pulmonary vessels.

Discussion

Data on the possible predisposing effects of mycotoxins in the manifestation of different infectious diseases are very limited. In production practices, FB₁ is usually combined with small quantities of other metabolites, such as FB₂ and FB₃, which are produced by the same fungus, *F. verticillioides*. The quantities of FB₂ and FB₃ metabolites are usually much lower, less toxic, and not deserving of the same attention relating to disease alteration as the main feed concentration of FB₁.^{9,23,33} The effect exerted by the fumonisin mycotoxins on the course and characteristics of the disease produced by *M. hyopneumoniae* and the interaction between the toxin and the bacterium have not been studied; however, in pigs, the lungs are the primary site of action of both components. Both agents occur frequently in pig production, and their presence may lead to severe economic losses, partly attributable to poorer production parameters. As an important causative agent of PRDC, *M. hyopneumoniae* may play a role in reducing the growth performance and body weight gain of pigs,²⁹ although the results of some studies, including this one, indicate that *M. hyopneumoniae* infection does not always cause this effect.^{5,30}

According to the literature, some *Fusarium* toxins may affect the performance of pigs in very low amounts; for example, T-2 toxin induces feed rejection, resulting in reduced body weight gain in a concentration as low as 0.5 mg/kg of feed.²⁴ On the other hand, FB₁ did not affect feed intake and body weight gain even when fed in much higher concentrations

(eg, 40 mg/kg of feed), despite the fact that the pigs ingesting it had developed rather severe pulmonary edema but no obvious clinical signs of pulmonary disease.³⁸ Another similar experiment conducted with growing pigs showed that dietary exposure from 1 to 10 ppm of FB₁ caused uneven body weight gain in the first 4 weeks of life, but growth stabilized between weeks 4 and 8. Dietary exposure to 10 ppm FB₁ reduced the body weight gain of boars by 10%.²⁷

The combined effect of FB₁ toxin and *P. multocida* serotype A was studied in experimentally infected piglets,¹⁴ and a significant decrease in the body weight gain was found only in piglets fed the toxin. In a previous study, simultaneous infection with *P. multocida* and *B. bronchiseptica* did not cause a significant reduction in feed intake or body weight gain, even when combined with dietary exposure to FB₁, despite the fact that FB₁ exposure resulted in a more severe bacterial infection.²³

The appearance of clinical signs characteristic of respiratory disease has been previously reported to occur on days 7 to 8 after experimental infection with *M. hyopneumoniae*,^{5,37} which was similar to findings in this study. The increase in body temperature noticed postinfection was probably due to the toxic metabolites produced by multiplication of the pathogen in the respiratory tract, the cytokines formed by the immune system, and damage to the epithelial cells.^{10,18} According to the results of previous studies, acute fumonisin toxicosis typically causes pulmonary edema, whereas chronic exposure (>6–8 weeks) at low doses (1–10 mg/kg of feed) results in pulmonary fibrosis. Generally, as in this study, these cases occur without recognizable clinical signs.⁴¹ Much higher doses of FB₁ (100–300 mg/kg of feed, or above 15 mg/kg of body weight) were required for expression of clinical signs.^{15,16}

M. hyopneumoniae infection on its own can produce lung lesions in growing pigs. CT images obtained 14 days postinfection (study day 44) showed appreciable changes in all infected animals. Dietary exposure to FB₁ aggravated the course of infection: the CT images obtained on study day 58 (day 28 postinfection) indicated progressive pulmonary disease in 2 of the 7 pigs infected with *M. hyopneumoniae* and fed FB₁ toxin (group MF).

The edematous changes and increased permeability of vessels provoked by FB₁ could facilitate the distribution of *M. hyopneumoniae* infection in the lung, resulting in further pathogen dissemination and aggravation of pneumonic damage, which could also change the typical macroscopic findings characteristic for *M. hyopneumoniae* infection. This process could be facilitated by the potent immunosuppressive effect of FB₁ on the humoral immune response in pigs, which was observed in recent studies at even lower (10 ppm) contamination levels of FB₁.³³ The interaction between *M. hyopneumoniae* and other respiratory pathogens has been previously studied, and many

Figure 8. (continued) by numerous neutrophils, lymphocytes, and desquamated epithelial cells in the bronchi/bronchioles, alveolar lumen, and/or septa. HE. **Figure 9.** Lung; pig, group MF. Accumulation of fibrin with numerous neutrophils, lymphocytes, and desquamated epithelial cells in the alveolar lumina, interalveolar septa, and interlobular tissue. HE.

demonstrated that pneumonia caused by mixed respiratory infections has a more severe clinical course.^{1,4,6,28,35,36,39}

By numerically expressing the CT lung lesions as average density values, a significant difference was clearly demonstrated between the *M. hyopneumoniae*-infected and uninfected pigs, but not between FB₁-exposed and unexposed pigs. The absence of a significant increase in the average lung density value in the pigs exposed to both FB₁ and *M. hyopneumoniae* infection could be due to the low numbers of experimental animals or, in part, because 1 MF group pig with the most severe lung lesions was euthanized before the end of study and its average lung density value was not included in the statistical analysis. Between 30 and 44 days of age, there was a significant change in the average lung density values in the 2 uninfected groups (C and F), which could presumably be due to the increased growth in lung size during this time. In the 2 infected groups (M and MF), however, no age-related difference was observed; in these groups, the HU values of x-ray absorption in the lungs did not change with age most likely due to the inflammatory processes produced.

Dietary exposure to 20 ppm of FB₁ resulted only in pulmonary edema, which was not demonstrated on the CT images and, therefore, caused no significant change in the average density values. However, the toxin did exert its effect at the cellular level, as indicated by the significantly elevated SA/SO value. At necropsy, pulmonary edema typical of the toxin could be seen in all of the animals exposed to fumonisins (groups F and MF).

Histopathologic findings in the lungs of *M. hyopneumoniae*-infected animals (group M) were very similar to those described in experimentally infected pigs,²⁶ with a progressive bronchointerstitial pneumonia from days 7 to 28, which became less severe at day 35. As in this study, previous authors²⁶ observed that infiltrating neutrophils and mononuclear cells were frequently seen interspersed between epithelial cells; lymphocytes, plasma cells, and neutrophils also were often observed within alveolar spaces, and the alveolar epithelium showed hypertrophy and hyperplasia of type II pneumocytes. From days 14 to 28, the expansion of alveolar septa by infiltrating plasma cells, lymphocytes, and macrophages was also observed.

Pathomorphological damages in the lung of pigs in this study fed FB₁ (group F) were typical for previously described fumonisin toxicosis in pigs, including interlobular edema of noninflammatory origin extending to the peribronchial, peribronchiolar, and perivascular regions of the lungs.^{8,16,40} Damages in the lung of pigs fed a diet containing FB₁ are probably due to the increased permeability of vessels, which is subsequently responsible for the perivascular and especially pericapillary edema observed.

In infected and toxin-fed animals (group MF), the histopathologic alterations typical to both *M. hyopneumoniae* infection and FB₁ effect had developed, which resulted in aggravation of the pneumonic process in 2 animals.

In summary, dietary exposure to FB₁ may complicate or facilitate the course of *M. hyopneumoniae* infection, as demonstrated by the progressive pulmonary disease observed in 2 pigs

and the severe clinical signs resulting in early euthanasia of another pig. Additional exploration of the effect exerted by the fumonisin toxin in the lungs and the apparent synergism between FB₁ and *M. hyopneumoniae* requires further studies.

Declaration of Conflicting Interests

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6. GENERAL DISCUSSION

Atrophic rhinitis caused by *B. bronchiseptica* and *P. multocida*, pneumonia induced by *M. hyopneumoniae*, and the respiratory diseases of swine elicited by multiple pathogens in the presence of predisposing factors, recently termed PRDC, cause huge economic losses to pig producers all over the world. Numerous studies have been conducted to explore the different features of the most important respiratory pathogens, but the role of mycotoxins present in feeds in facilitating colonisation by microorganisms and in the development of syndromes jointly produced by microorganisms and mycotoxins have been studied by few researchers only. Halloy et al. (2005) studied the swine respiratory disease brought about by the interaction of FB₁ toxin and *P. multocida*, while Ramos et al. (2010) investigated the predisposing role of FB₁ toxin in pigs experimentally infected with PRRS virus. The role of FB₁ toxin in dual infection by *B. bronchiseptica* and *P. multocida* or in the case of *M. hyopneumoniae* infection has not been studied so far, and nor have the interactions among these agents been investigated. By the piglet-rearing method used by us we could prevent the colonisation of experimental animals by respiratory pathogens, which enabled us to study the pathogenesis of pulmonary lesions induced by respiratory pathogens experimentally introduced into the pigs' respiratory tract. In addition, we studied the influence of FB₁ toxin concentrations exceeding the maximum permissible limit of 5 mg/kg of feed (EU Commission Recommendation, 2006) but often occurring under field conditions (10 mg/kg and 20 mg/kg of feed) on the type or development of lung lesions. In feeds used under field conditions, FB₁ very often occurs in combination with other fumonisin metabolites (e.g. FB₂ and FB₃) produced by the same fungal species, *F. verticillioides*, which, however, may have a much less important role in the development of diseases than FB₁ (Fodor et al., 2006; Stoev et al., 2012). Acute FB₁ toxicosis typically induced pulmonary oedema in pigs, while chronic (>6- to 8-week) exposure to low doses of FB₁ (1–10 mg/kg of feed) resulted in the development of pulmonary fibrosis, in most cases not manifested in clinical signs (Zomborszky-Kovács et al., 2002). FB₁ toxin causes clinical signs when present in doses higher than the above (100–300 mg/kg of feed, and >15 mg/kg of body weight) (Harrison et al., 1990; Haschek et al., 1992).

The role of FB₁ in facilitating bacterial infections can be supposed because of the immunosuppressive property of the toxin, which might be related to the accumulation of free sphingoid bases, which has been reported to inhibit the proliferation of lymphocytes (Taranu et al., 2005). Oswald et al. (2003) have demonstrated that FB₁ taken up with the feed decreased resistance to *Escherichia coli*, resulting in increased colonisation of the small intestine by these bacteria.

Stoev et al. (2012) reported reduced antibody production after vaccinating pigs treated with FB₁ toxin against Aujeszky's disease.

Similarly, Halloy et al. (2005) suggested that the predisposing effect of FB₁ toxin to *P. multocida* infection was attributable to the impaired immune response and the reduced phagocytic ability of pulmonary macrophages. The increased sensitivity of pulmonary capillaries to FB₁ may also enhance the susceptibility of the lungs to infection (Halloy et al., 2005). The proinflammatory effect of FB₁ toxin has also been demonstrated by several studies (Taranu et al., 2005).

Ramos et al. (2010) could also demonstrate the predisposing effect of FB₁ toxin in pigs experimentally infected with PRRS virus and treated with FB₁ toxin, also attributing this effect to the immunosuppressive action of the toxin.

M. hyopneumoniae, as one of the major causative agents of PRDC, plays a role in decreasing the production and body weight gain of pigs (Sibila et al., 2009); however, according to some studies the effect of *M. hyopneumoniae* infection is not always significant in this regard (Clark et al., 1993; Sitjar et al., 1996). Also in the case of *B. bronchiseptica* and *P. multocida*, the two other bacteria studied by us and playing an important role in the aetiology of PRDC, there are conflicting reports in the literature regarding their effect on body weight gain. The negative effect of *B. bronchiseptica* and *P. multocida* infection on body weight gain has been demonstrated by multiple studies (Giles et al., 1980; Underdahl et al., 1982; Cowart et al., 1990; Hall et al., 1990; Brockmeier et al., 2000). In several cases, however, the significance of this effect could not be proved (Tornøe and Nielsen, 1976; Rutter et al., 1984; Straw et al., 1984). According to certain data of the literature, *Fusarium* toxins may affect the production of pigs already in low doses, e.g. T-2 toxin causes feed refusal resulting in decreased body weight gain already in a dose of 0.5 mg/kg of feed (Rafai et al., 1995). In their experiment with pigs, Tóth et al. (2000) demonstrated that the feeding of FB₁ toxin in a dose of 40 mg/kg of feed had no marked influence on feed intake and body weight gain, despite the fact that the animals ingesting the toxin developed relatively severe pulmonary oedema which, however, was not yet manifested in clinical signs. Rotter et al. (1996) studied the effects of purified FB₁ added to the diet of pigs at doses of 0.1, 1 and 10 mg/kg of feed, respectively, and demonstrated a sex-related difference in sensitivity, with gilts being less sensitive. While in male piglets the body weight gain measured in weeks 4–8 decreased by 10% in linear correlation with increasing concentrations of the toxin, in gilts there was no detectable difference. Body weight gain showed marked fluctuation in both sexes in the first 4 weeks but became much more balanced in the second 4-week period.

In studies investigating the combined effect of FB₁ toxin and *P. multocida* (Halloy et al., 2005) or FB₁ toxin and PRRS virus (Ramos et al., 2010) in experimentally infected piglets, in both cases a significant decrease in body weight gain was found in the case of infected pigs fed the toxin. In our own experiments, we did not find significant differences ($p < 0.05$) in the average body weight of the different groups on the individual days of the study (Experiments 1–3), although the growth of pigs in the groups subjected to infection combined with FB₁ toxin treatment was inferior to that of animals in the other three groups (Experiments 2–3).

In our studies, we successfully confirmed the previous statement that *B. bronchiseptica* infection could induce lung lesions in young piglets also on its own (Meyer and Beamer, 1973; Brassine et al., 1976; Janetschke et al., 1977; Underdahl et al., 1982). Mild clinical signs (mild serous nasal discharge, sneezing, wheezing during breathing and hoarse voice) appeared already after *B. bronchiseptica* infection (Brassine et al., 1976; Brockmeier et al., 2002a). However, *B. bronchiseptica* infection not accompanied by clinical signs was also reported under experimental conditions (Tornøe and Nielsen, 1976). In our studies, early colonisation of the lungs by *B. bronchiseptica* could be demonstrated by CT (Experiments 1–2), as CT examination performed on day 12 after experimental infection demonstrated already well-recognisable diffuse pathological lesions extending to several lobules of the lungs in 97% (Experiment 1) and 57% (Experiment 2) of the infected animals.

Whittlestone et al. (1972) were the first to describe the lung lesions caused by *M. hyopneumoniae* as an independent aetiological factor in growing piglets under experimental conditions. Such lesions were subsequently reproduced in several other experiments (Lorenzo et al., 2006; Redondo et al., 2009). This was supported also by our studies, as CT examination performed 14 days after experimental infection showed lung changes demonstrable on the CT scans in 100% of the infected animals (Experiment 3).

The interactions among *B. bronchiseptica*, *P. multocida*, *M. hyopneumoniae* and other respiratory pathogens have already been studied in several previous experiments, which demonstrated that pneumonia induced by mixed respiratory infections had a more severe course (Yagiashi et al., 1984; Ciprian et al., 1988; Dugal et al., 1992; Amass et al., 1994; Chung et al., 1994; Shibata et al., 1998; Thacker et al., 1999, 2001b; Desrosiers, 2001; Halloy et al., 2004). In our second experiment, *P. multocida* infection performed on day 12 after *B. bronchiseptica* infection and the ingestion of FB₁ toxin with the feed notably aggravated the clinical signs: the pigs started to cough, and the animals that eventually died ($n = 3$; $n = 2$ in the infected and toxin-treated group, $n = 1$ in the infected group) had developed severe dyspnoea prior to death. Prolonged fever developed in the infected groups, as a result of infection, from day 22: it was the

most pronounced in Group D in which some pigs had body temperatures exceeding 40.0 °C over a period of several days. The progressive nature of pneumonia was demonstrated by the third CT examination performed on day 25, when already 71% of the pigs in the infected groups (57% in Group C and 86% in Group D) showed pathological lesions in their lungs, and the lesions had become more extensive and assumed a focal character. By the end of the experiment (day 39) the severity of the clinical signs had decreased and the body temperature of several animals had returned to normal. The CT scans taken at that time showed that pneumonia had become more extensive and its focal nature was more pronounced than on day 25. The gross pathological examinations confirmed the localisation and extent of the lung lesions seen on the CT scans. The prevalence, extent and severity of lung lesions were the highest in Group D, which is consistent with the results reported by Halloy et al. (2005), according to which *P. multocida* infection produces more severe and more extensive pneumonia in the presence of FB₁ toxin (Experiment 2).

According to data of the literature, the appearance of clinical signs characteristic of respiratory disease can be expected on days 7–8 after *M. hyopneumoniae* infection (Clark et al., 1993; Thacker, 2006), which was confirmed also by our Experiment 3. The elevation of body temperature after infection may have been due to the toxic metabolites produced by the pathogen during its multiplication in the epithelial cells of the respiratory tract after colonisation, the cytokines produced by the immune system, and the epithelial cell damage (Fossum et al., 1998; Lorenzo et al., 2006). Together with the appearance of clinical signs, a body temperature exceeding 40.0 °C was measured in the infected animals over a period of several days. During Experiment 3, only a single animal died; this death occurred in the infected and toxin-treated (MF) group. The findings of the gross pathological examinations were consistent with the results of the CT scans in terms of the localisation and extent of the lung lesions. Expressing the lung lesions in numerical terms using the mean density values, we demonstrated a significant difference between the infected and the uninfected pigs. Between days 30 and 44 of life, the pigs' age significantly influenced the mean density values. This was presumably due to the change in lung size. In two groups of infected animals, however, no age-related change could be observed; in these groups the HU value of the lungs did not change with age because of the inflammatory processes induced by the infection. There was no significant difference between Group M and Group MF; however, it should be taken into account that in Group M a pig showing extensive lesions died and, thus, its mean density value was not included in the statistical evaluation. Dietary exposure to FB₁ toxin aggravated the course of infection, as analysis of the CT scans taken on day 58 (corresponding to day 28 after infection) showed improvement in five pigs but a

progressive process in two infected pigs that were fed FB₁ toxin.

In Experiment 3, gross pathological and histopathological changes typical of both pathological factors were demonstrable in pigs treated with FB₁ toxin and infected with *M. hyopneumoniae*. In the pigs experimentally infected with *M. hyopneumoniae*, we also observed lesions similar to those reported by Redondo et al. (2009) earlier. In addition to bronchointerstitial inflammation developing in the lungs, other changes included marked neutrophil granulocytic and mononuclear cell infiltration among the epithelial cells, appearance of an exudate containing lymphocytes, plasma cells and neutrophil granulocytes in the alveoli, as well as hypertrophy and hyperplasia of the alveolar epithelial cells (type II pneumocytes). Besides the characteristic picture of catarrhal bronchointerstitial pneumonia attributable to the effect of *M. hyopneumoniae*, mild hyperaemia appeared in several internal organs due to the circulatory insufficiency developing in the lungs. In the treated animals, exposure to FB₁ resulted in oedema of inflammatory origin in the peribronchial, peribronchiolar and perivascular space due to altered permeability of the blood vessels, as described also by other authors (Fazekas et al., 1998; Haschek et al., 1992; Zomborszky-Kovács et al., 2000). Mild perivascular oedema was visible also in organs other than the lungs (in the brain and the kidneys), mainly around the capillaries. Of the different mycotoxins, ochratoxin was found to cause the most pronounced renal damage; however, certain authors have reported similar, although somewhat milder, renal degeneration as a result of exposure to FB₁ toxin (Bucci et al., 1998; Voss et al., 2001; Howard et al., 2001). Furthermore, a causal relationship has been demonstrated between fumonisin and nephropathy occurring in humans and animals both in the Balkans (Stoev, 2010a) and in South Africa (Stoev, 2010b). In histological sections, we also found mild degenerative changes in epithelial cells of the convoluted tubules of the kidneys.

7. CONCLUSIONS

By the piglet-rearing method applied in the study we could prevent the infection of experimental animals by respiratory pathogens, and thus we could study the pathogenesis of lung lesions produced by respiratory pathogens introduced into the respiratory tract by experimental infection.

We successfully demonstrated that both *B. bronchiseptica* and *M. hyopneumoniae* could cause pneumonia in young and growing piglets also when used alone (in the form of monoinfection).

In our experiments, we did not find significant differences ($p < 0.05$) among the groups in average body weight gain (Experiments 1–3), although in the groups subjected to experimental infection combined with FB₁ toxin treatment the growth of pigs was inferior to that seen in the other three groups (Experiments 2–3).

Based on the results of Experiment 2, it can be established that in pigs infected by *B. bronchiseptica* and *P. multocida* the consumption of FB₁ toxin in a concentration of 10 mg/kg of feed increased the probability of development of pneumonia, as the number of pigs with lung lesions was higher in groups fed FB₁ toxin.

In addition, it can be stated that in pigs subjected to dual infection by *B. bronchiseptica* plus *P. multocida* or to infection by *M. hyopneumoniae*, the consumption of FB₁ toxin in a concentration of 10 mg/kg or 20 mg/kg of feed aggravated the course of bacterial diseases, as the clinical signs were the most pronounced in the experimentally infected groups fed FB₁ toxin, and in these groups mortality also occurred.

We have elaborated a possible application of computed tomography for studying the time-course of development and the pathogenesis of pneumonia. This method may provide useful information for the study of other respiratory diseases as well. A great advantage of computed tomography is that it enables us to monitor the localisation, extent and character of the developing pathological lung lesions in live animals, simultaneously with the appearance of the disease. By the evaluation of CT scans taken at different time-points we can obtain new scientific insights into the pathogenesis of different respiratory diseases or, in the framework of applied experiments, we can compare the efficacy of therapeutic interventions on the disease entities concerned.

By applying a new model for the evaluation of lesions developing in the lungs, in Experiment 3 we expressed the mean density values in numerical terms and demonstrated a significant difference between the infected and the non-infected animals.

The mean density calculated from the cumulative pixel frequency values collected from selected areas of the lungs was significantly influenced by the treatment applied and the age (the time of examination), with an interaction being demonstrable between the two effects. The mean density decreased significantly between days 30 and 44 ($P < 0.01$) but it did not change between days 44 and 58. The mean density of the lungs of the infected groups was 5–10% higher than that of the non-infected pigs ($P < 0.001$). In the infected animals, the mean density values obtained on days 44 and 58 did not differ significantly from the values found on day 30. However, using the numerical model we could not demonstrate a significant effect induced by FB_1 treatment either in the infected or in the non-infected group.

8. NEW SCIENTIFIC RESULTS

1. CT could be applied and a useful tool for studying the pathological conditions in the lower respiratory tract of swine.
2. Elaboration of a new method for the examination of the lung of swine with CT as well as for the evaluation of the images that made the quantitative analysis of the pathological processes possible.
3. *B. bronchiseptica* mono-infection was able to produce lung lesions in young pigs.
4. In case of dual infection by *B. bronchiseptica* and *P. multocida*, dietary exposure to the mycotoxin FB₁ above the advised level of 5 mg/kg of feed raised the risk of pneumonia and increased the extent and severity of the pathological changes produced.
5. Dietary exposure to FB₁ may facilitate or complicate the course of *M. hyopneumoniae* infection, as demonstrated by the progressive pulmonary disease observed in 2 pigs and the severe clinical signs resulting in early euthanasia of another pig.

9. SUMMARY

As one of the most prevalent respiratory pathogens, *M. hyopneumoniae* has played a decisive role in the aetiology of respiratory diseases for several decades, usually in association with other pathogenic bacteria (*A. pleuropneuminae*, *B. bronchiseptica* and *P. multocida*), resulting in a complex respiratory syndrome. The prevalence of other respiratory pathogens varies more widely; however, over the past 20 years PRRS virus, porcine circovirus type 2 and the newly emerging swine influenza virus strains have caused the biggest problems to swine health professionals in the leading pig-producing countries of the world.

At the Department of Physiology and Animal Hygiene of Kaposvár University, in cooperation with the Institute of Diagnostic Imaging and Radiation Oncology of the same University, animal experiments aimed at monitoring the pathological processes that take place in the lungs of pigs have been conducted since 1997. During these experiments, the basic techniques of using modern diagnostic imaging procedures (CT, MRI) for the above purpose were developed.

In the experiments carried out in the framework of my doctoral work, we studied the pneumonia induced in young piglets by *B. bronchiseptica* (Experiment 1), the correlations among *B. bronchiseptica*, toxigenic *P. multocida* and FB₁ toxin (Experiment 2), as well as those between *M. hyopneumoniae* and FB₁ toxin (Experiment 3) in the production of lung lesions in pigs.

Our applied CT studies were performed using the only CT scanner currently available in Hungary for studies of such type, but being one of the most advanced such machines existing at present (SIEMENS Somatom Emotion 6 multislice CT scanner), at the Institute of Diagnostic Imaging and Radiation Oncology of Kaposvár University, according to routine technical principles. The examinations were carried out on anaesthetised live animals at multiple points of time, and were focused on studying lesions in the lower respiratory tract.

The day when the piglets arrived was day 0 of the experiment. After early weaning, the piglets were reared artificially in disinfected, isolated rooms, and were fed a milk replacer diet from an automatic milk-powder mixer and feeder up to day 16 of life and a dry coarse meal from day 7 of life up to the end of the experiment.

Experiment 1

The experiment included 30 female piglets of 3 days of age, placed in two separate rooms, according to the following experimental design: Group A – non-infected control piglets (n = 10) and Group B – piglets infected with *B. bronchiseptica* (n = 20). Experimental infection with *B. bronchiseptica* was done by intratracheal inoculation (strain KM22, 10⁶ CFU/ml) on day 4 of the experiment, after the intact condition of the lungs had been checked by CT. Subsequent CT scans were taken on days 16, 25 and 39 of the experiment. At the end of the experiment (on day 39), the piglets were killed by bleeding after narcosis and necropsied according to the rules of the profession, and the lung lesions were recorded. During the experiment, there were no significant differences between the treatment groups in terms of body weight gain. On day 4 after infection, sneezing, stertorous breathing and mild coughing accompanied by mild serous nasal discharge could be observed in Group B. CT scans taken on day 16 after infection confirmed colonisation of the lungs of young piglets by *B. bronchiseptica*, as pathological lesions were found in 95% of the infected animals. The gross pathological findings were consistent with the changes seen on the CT scans and confirmed their localisation. Lesions were primarily seen in the cranial and middle lobes as well as in the cranial third of the caudal lobe. The affected lungs exhibited acute catarrhal inflammation with areas showing signs of chronicity and mottled appearance due to haemorrhages, with pleuritis occurring as a secondary complication in some animals.

Experiment 2

This experiment included 28 female piglets of 3 days of age. A total of four groups, each comprising 7 piglets, were formed and housed in two separate rooms: Group A – non-infected group not treated with FB₁ toxin, control animals; Group B – non-infected group treated with FB₁ toxin; Group C – group infected only (combined infection with *B. bronchiseptica* and *P. multocida*); and Group D – infected animals (combined infection with *B. bronchiseptica* and *P. multocida*) also treated with FB₁ toxin.

Piglets of Groups C and D were infected with *B. bronchiseptica* (strain KM22, 10⁶ CFU/ml) on day 4 of the experiment and with *P. multocida* (strain LFB3) on day 16 of the experiment. Piglets of Groups B and D consumed a diet containing 10 ppm FB₁ from day 16 up to the end of the experiment. CT scans were taken on days 4, 16, 25 and 39 of the experiment. At the end of the experiment (on day 39) the piglets were killed by bleeding after narcosis and necropsied according to the rules of the profession, the lung lesions were recorded and samples

were taken and processed for histopathological examination. During the experiment, no significant differences were observed between the treatment groups in terms of body weight gain.

During the experiment, a total of three piglets died, none of them due to causes associated with their rearing. One piglet of Group C died one day after infection with *P. multocida*. Two piglets of Group D died 8 and 18 days, respectively, after *P. multocida* infection. During postmortem examination, bronchopneumonia of varying severity, acute catarrhal inflammation with areas showing signs of chronicity and mottled appearance due to haemorrhages, with pleuritis as a secondary complication, were found in the 3 pigs that died and in 5 other animals (2 pigs of Group C and 3 pigs of Group D).

Analysis of the CT scans demonstrated lung lesions in 3 pigs of Group C and 5 pigs of Group D on day 16 of the experiment (at the second CT examination). Lesions indicative of pneumonia were much more marked on day 25 of the experiment (at the time of the third CT examination), 9 days after *P. multocida* infection and the start of FB₁ toxin feeding. After *P. multocida* infection, lesions were found only in one piglet each of Groups C and D. Two piglets (one piglet each of Groups C and D) showed mild changes after *B. bronchiseptica* infection; however, these changes were no longer demonstrable at the end of the experiment either on the CT scans or during necropsy. The lesions developing after *B. bronchiseptica* infection were of diffuse character, extending to entire lobes or lobules, whereas those induced by *P. multocida* infection were smaller, well demarcated and of focal nature.

B. bronchiseptica infection could induce lung lesions in young piglets alone (as monoinfection). Infection with *P. multocida* and the consumption of FB₁ toxin aggravated these lesions. The mortality was the highest and the observed lung lesions were the most severe and most extensive in Group D.

As a conclusion, it can be established that dual infection with *B. bronchiseptica* and *P. multocida* combined with the consumption of FB₁ toxin increases the likelihood of pneumonia developing in pigs and increases the extent and severity of the lesions induced.

Experiment 3

This experiment included 28 female piglets of 3 days of age. A total of four groups, each comprising 7 piglets, were formed and housed in two separate rooms: Group A – non-infected group not treated with FB₁ toxin, control animals; Group B – non-infected group treated with FB₁ toxin; Group C – group infected only (infection with *M. hyopneumoniae*); and Group D – infected

animals (*M. hyopneumoniae* infection) also treated with FB₁ toxin. The feeding of the diet containing 20 mg FB₁ toxin per kg of feed was started on day 16 (Groups B and D), and infection was performed on day 30 (Groups C and D; strain Mp 49, 3×10^5 CFU/ml). CT scans were taken on days 30, 44 and 58 of the experiment. At the end of the experiment (on day 58) the piglets were killed by bleeding in narcosis and necropsied according to the rules of the profession, then the lung lesions were recorded and samples were taken and processed for histopathological examination.

During the experiment, we did not find significant differences among the treatment groups in body weight gain. After infection (from postinfection day 31) elevated body temperature (39.5–40.8 °C) occurred in Groups C and D, and from day 37 clinical signs (coughing, sneezing, hoarse voice, dyspnoea) could be observed. During the experiment, a single death occurred in the infected and toxin-treated group. On the CT scans taken 14 days after infection, well-visible lung changes indicative of inflammation were observed in all piglets of the two infected groups. These lesions initially occurred around the smaller airways, but subsequently they increased in size and at the end of the experiment (on day 58) they were found to be extensive in several animals. The most severely affected parts of the lungs were the cranial parts of the lung lobes. At necropsy, lung areas showing acute and subacute catarrhal inflammation were found in the affected animals with an extension identical with that seen on the CT scans.

Mycoplasma hyopneumoniae infection was able to induce lung changes in the growing piglets even in itself (as mono-infection). However, the ingestion of FB₁ toxin aggravated these lesions. Both the mortality rate and the severity and extent of the lung lesions observed were the highest in Group D. From the results, it can be concluded that *M. hyopneumoniae* infection combined with the consumption of FB₁ toxin raises the probability of pneumonia developing in the pigs and increases the severity and extent of lung lesions.

The CT scans taken of lungs kept at a specific pressure during the time of CT examination proved to be suitable for detecting the lung lesions. Using the measurement method elaborated by us, we could demonstrate a significant difference between the infected and the non-infected pigs in the mean density values of the lungs. The feeding of a diet containing 20 ppm FB₁ toxin induced only histopathological changes and mild macroscopic lesions such as oedema in the lungs of pigs in the treated groups. By analysing the CT scans, oedema of such minor extent could not be expressly detected.

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12. PUBLICATIONS & PRESENTATIONS

SCIENTIFIC PAPERS AND LECTURES ON THE SUBJECT OF THE DISSERTATION

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Pósa R., Kovács M., Szabó-Fodor J., Mondok J., Bogner P., Repa I., Magyar T. Effect of *Mycoplasma hyopneumoniae* and fumonisin B₁ toxin on the lung in pigs. *Italian Journal of Animal Science*. 2009. 8(3):172-174.

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13. CURRICULUM VITAE

Dr. Roland Pósa was born in Zenta, then Yugoslavia, on 12 March 1978. He attended primary school at Orom and secondary school in Bácsstopolya.

In 2004 he obtained his doctor of veterinary medicine (DVM) diploma at the Faculty of Veterinary Science, Szent István University, with '*cum laude*' qualifications.

Between 2006 and 2009 he was a full-time student at the Doctoral School of Animal Science of Kaposvár University.

From February 2005, he participated in the theoretical and practical teaching of the subjects anatomy, histology and physiology at the Department of Physiology and Animal Hygiene, Faculty of Animal Science of Kaposvár University, and in the research activities of the Department.

From May 2005, he participated as a veterinarian in the animal experiments related to research conducted at the Department of Animal Nutrition, Faculty of Animal Science of Kaposvár University.

From April 2006 he worked as a private veterinarian in Somogy County.

In 2007 he successfully passed a basic examination in public administration.

In 2008 he established a family farm in Szenna, which he has been continuously developing and operating ever since.

In 2013 he passed a basic examination in Agricultural Special Consulting.

Since 2002 he has been an active member of the World Organisation of Hungarian Veterinarians, and currently he is a Board member of the Organisation.

Since 2008 he has been an organiser of the Farmers' Circle of Zselic.

Since April 2011 he has been the chairman of the Somogy County Organisation of the Smallholders' Civil Association.

Since February 2013 he has been a national delegate of the Somogy County organisation of the Hungarian Chamber of Agriculture, Food and Rural Development and a member of the Animal Breeding, Fisheries Management and Dairy Processing Department of the Chamber.

Command of foreign languages:

High-level command of Serbian

Base-level command of English