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Enzootic pneumonia of pigs – diagnostic notes (review)

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Abstract

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The main purpose of this review is to analyze the methods used to diagnose the enzootic pneumonia which is one of the most significant and widespread respiratory infections in swine. This is a necessary condition so that adequate and timely measures for prevention and control of the disease can be identified. The benefits include decreasing economic losses in industrial pig farming. Discovering *M. hyopneumoniae* as a respiratory disease can be disappointing, as it isn't always connected with the development of symptoms. The diagnosis of enzootic pneumonia requires a complex approach, i.e. combining clinical symptoms and lung lesions with results from various laboratory analyses. Depending of the stage which the disease has reached, and the type of probes analyzed, various clinical tests can be applied. Those tests are typically sensitive and specific and can be used for accurate diagnosis.

Keywords: pigs; Enzootic pneumonia; *Mycoplasma hyopneumoniae*; diagnosis

Introduction

Respiratory infections carry a health and economic threat for industrial pig farming. Such infections often affect heavy growing pigs and those in early stages of fattening (Vicca et al., 2002; Sibila et al., 2008; Maes et al., 2011; Pieters et al., 2014). Sometimes the disease develops as mono infections, and sometimes as polyethiological basis, which can lead to Porcine Respiratory Disease Complex – PRDC (Bochev, 2007; Del Pozo Sacristán, 2014; Chae, 2016). One of the main and permanent etiological factors in PRDC is *Mycoplasma hyopneumonae* (*M. hyopneumonae*), the cause of enzootic pneumonia (EP) in pigs (Opriessnig et al., 2004; Thacker, 2006; Pepovich, 2015). The disease is widely spread in all countries with intensive pig farming, including in Bulgaria. Enzootic pneumonia is one of the more significant respiratory diseases in pigs and leads to major economic losses due to high morbidity, bad fodder conversion, decreased average daily gain, reduced growth, increased costs for therapy and prophylactic, increased mortality (Georgakis et al., 2002; Maes et al., 2008; E. Thacker & B. Thacker, 2012; Pepovich, 2015). All those factors make the disease a serious health concern for the veterinary practice and science. This in turn raises the necessity of diagnostics and identifying timely and adequate measures for prevention and control.

Diagnosis of enzootic pneumonia in pigs is based on:

- Clinical signs;
- Pathological (macroscopic and microscopic) lesions;
- Determining the causing agent (through isolating – bacteriological study; through antigen detection – immunofluorescence or immunohistochemistry; through proving DNA – PCR);
- Finding specific antibodies.

Clinical signs

Dry non-productive cough is common in enzootic pneumonia. It starts most often around 2 weeks after the infection and reaches its peak after 5 weeks (Kobisch et al., 1993; Thacker, 2001). The cough is strongest when the pigs are physically active in the early morning hours, during feeding. Other side effects include depression, febrility (40.5ºC), dispnea, tachycardia, nasal secretion, conjunctivitis, bad fod-

der conversion, growth reduction (Morris et al., 1995; Sibila et al., 2009; Pepovich et al., 2014). Some authors (Yeske, 2003) classify the intensity of coughing, which is important for diagnosis, using a point based scale – 0 for the lack of coughing during morning activities, 1 – sporadic coughing in less than 10% of pigs, 2 – coughing in 10-15% of pigs and continuing during physical activities and 3 – persistent coughing in more than 50% of pigs. According to the severity of different clinical symptoms Tazayan (2009) proposes three clinical forms of the disease – subclinical, acute and chronical. One of the most frequent symptoms can be found in Table 1. The severity of symptoms depends not only on the virulence but also from the presence of secondary infections and environment factors (Leon et al., 2001; Villarreal, 2010).

Table 1. Frequency of the symptoms in enzootic pneumonia, caused by *M. hyopneumoniae* (%) (Tazayan, 2009)

Clinical symptoms	Clinical forms		
	Subclinic	Acute	Chronic
Weigh loss	5	89	100
Depression	10	65	100
Dispnea	3.5	44	100
Stridors	–	18	100
Nasal secretion	–	10	95
Tachycardia	–	76	85
Cough	–	55	89
Temperature raise	–	30	63
Conjunctivitis	–	15	39
Arthritis	–	1	6
Cachexia	–	–	87

Pathological lesions

Macroscopic changes

Pathological changes in enzootic pneumonia are typical and can be identified primarily in the lungs of 20 to 80% of slaughtered pigs (Sarradell et al., 2003; Leneveu et al., 2005; Sibila et al., 2007; Pepovich et al., 2017). Macroscopic changes are characterized with catarrhal bronchopneumonia which affects the apical, cardiac, diaphragmatic and part of the intermediate lobes of the lungs (Sims & Glastonbury, 1996; Pepovich et al., 2017). More often the damage spreads in the intermediate, left cardiac and apical lobes compared to the diaphragmatic lobes which are affected less frequently (Maes et al., 1996). During the acute phase of EP, the affected areas are well segregated from normal tissue with presence of a catarrhal exudate in the airways, a pale purple colour of the parenchyma of the lung and a „meaty" consistency (Christensen et al., 1999; Del Pozo Sacristán, 2014; Pepovich et al., 2017). When the disease becomes chronic, the lesions become deeper red, with better demarcation and limited in exudate quantity in the respiratory tract (Figure 1). The bronchial and mediastinal lymph nodes are also affected. They are edematically enlarged and hyperemic (Vicca, 2005; Pepovich et al., 2017).

The extent of pulmonary damage from *M. hyopneumonae* Kristensen et al. (2014) define through assessing the percentage of lung lesions from the surface of the lungs as follows: left apical lobe (5%); left cardiac lobe (5%); left diaphragmatic lobe (30%); right apical lobe (10%); right cardiac lobe (10%); right diaphragmatic lobe (35%) and right accessory lobe (5%).

Microscopic changes

The histological changes in enzootic pneumonia in pigs depend on the stage of the disease. According to Maes et al. (1996) the biggest histological changes in experimental infection are seen after 8 weeks, but after about 4 months

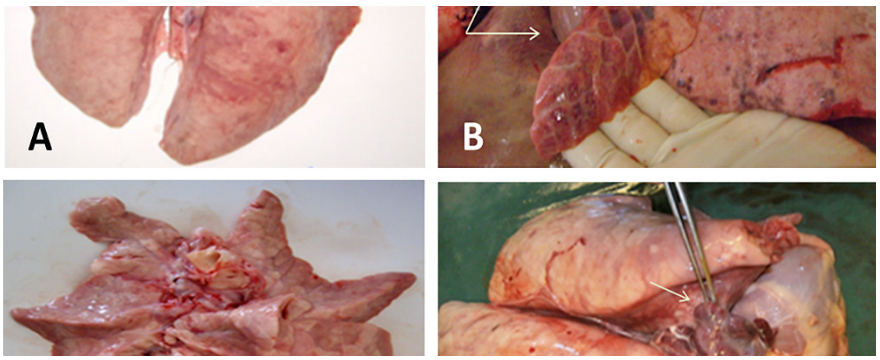

Fig. 1. Lung on pig died from acute enzootic pneumonia (A). Affected left apical and cardiac lobes of the lung with chronic enzootic pneumonia (B) (Pepovich et al., 2017)

they begin to disappear. During the acute phase of the disease neutrophilic exudate in the alveoli with minimal lymphoid hyperplasia is found (Sims & Glastonbury, 1996; Pepovich, 2015; Pepovich et al., 2017). The chronic phase of EP is characterized with lymphoid hyperplasia with increased amount of lymphocytes in peribronchial, peribronchiolar and perivascular areas and with thickening of the interalveolar partitions (Ross, 1999; Pepovich, 2015; Pepovich et al., 2017).

Pathohistological changes in EP could be classified regarding their characteristic and severity, as a result of which Calsamiglia et al. (2000) develop modified classification the lesions from 0-4. The lack of changes 0. Score 1 includes inflamation changes (interstitial pneumonia or purulent bronchopneumonia). Score 2 includes moderate or intensive infiltration of macrophages or lymphocytes with exudative process present in the respiratory system. Scores 1 and 2 are not specific for EP. Score 3 is presented by peribronchial lymphoplasmatic hyperplasia, edema in the alveolar area with neutophils, macrophages and plasma cells. Score 4 includes the changes described in the score 3, along with peribronchial and perivascular lymphoid nodules. Scores 3 and 4 include changes that are typical but not specific for pneumonia caused by *M. hyopneumoniae* (Thacker et al., 2001).

The link between the macroscopic and microscopic lung lesions is difficult to apprise. While the macroscopic lesions evaluate the extent damages, the microscopic ones tell the specificity and severity of the examined lung fragment. Lung samples which show histopathological lesions compatible for EP have been seen, without any macroscopic changes. Probably these microscopic lesions are too local, which cannot be established macroscopically (Sibila, 2004).

Determining of the causal agent

Microbiological studies

M. hyopneumoniae is characterized by slow multiplication *in vitro* (10–30 days) and high demands on the food environments compared to *M. hyorhinis* and *M. hyosynoviae,* which are less demanding in cultivation (Shafiev & Kudryashov, 2002). For its multiplication *M. hyopneumoniae* demands cholesterol, pH around 7.0 and optimal temperature 36–37°C. It develops in environments containing trypsin lysate from calf hearts as well as normal horse serum (as a source of cholesterol), yeast extract, glucose, penicillin for inhibiting the growth of side flora etc. (Mitov, 2000). On solid food environments *M. hyopneumoniae* forms distinctive small, hemispherical colonies with sizes from 0.6 to 4 mm like "fried eggs" with a solid raised center and a loose lace periphery. On liquid food environments *M. hyopneumoniae* causes a slight turbidity of the culture which is taken into account by visual observation. Micoplasms have no proteolytic qualities and don't hydrolyze urea. Most break down glucose or arginine as well as fructose, maltose, glycogen, dextrin and starch (Popova, 2009). As noted by Wallgren et al. (1994) and Strait et al. (2004), at the first isolation of the causative agent of enzootic pneumonia of pigs, its growth on food environments is outpaced by saprophyte and other strains of micoplasms that don't have etiological significance.

Although the microbiological method is considered as „gold standard" at the identification of this microorganism, due to the slow and demanding growth of *M. hyopneumoniae*, it finds no great practical importance in the laboratory practice of many countries (Thacker, 2004; Sibila et al., 2009).

Immunofluorescents (IFA) and Immunohistochemestry (IHC)

The immunofluorescent method (IFA) is considered to be specific for the discovery of *M. hyopneumoniae* in lung tissue but only at the acute stage of the illness, when there is a great number of micoplasms (Ross, 1999). The main flaw of these techniques is that the diagnosis is determined after the animals have been slaughtered. Other flaws are the artefacts, which form during the processing of the tissue sections (freezing) and the use of polyclonal antiserums could lead to non-specific finds of other types of micoplasms connected to pigs (Cheikh Saad Bouh et al., 2003). All of this limits the use of IFA at the diagnostic laboratories.

Immunohistochemistry (IHC) uses paraffin-embedded lung tissue fixed in formalin. The reaction is sensitive and specific as it proves the desired antigen and the typical lesions characteristic of a particular disease (Halbur, 1997). For the detection of *M. hyopneumoniae* antigens and for the determination of cell populations in the present pulmonary lesions of pigs infected with *M. hyopneumoniae,* also monoclonal and polyclonal antibodies find use in IHC (Sarradell et al., 2003; Rodríguez et al., 2004).

Molecular biology methods (PCR)

Distinctive features of the method are: its ability to determine the presence of the pathogen until the formation of specific antibodies; much faster in comparison to the cultural method; highly sensitive and specific method; can be used both for life diagnosis and *post mortem;* a diverse set of samples can be tested (Calsamiglia et al., 1999; Ruiz et al., 2002).

The first use of PCR for diagnosing enzootic pneumonia in pigs was in 1991 (Harasawa et al., 1991). Later the method was modified and used in different variations: single PCR

(Harasawa et al., 1991; Sorensen et al., 1997; Caron et al., 2000; Pepovich, 2015); multiplex PCR (Caron et al., 2000); nested (nPCR) (Calsamiglia et al., 1999; Verdin et al., 2000; Kurth et al., 2002; Pepovich, 2015); PCR with an internal control (Verdin et al., 2000; Kurth et al., 2002).

Different pathologic materials could be used for diagnostics: nasal samples (Caron et al., 2000; Verdin et al., 2000; Kurth et al., 2002; Ruiz et al., 2002; 2003; Vicca et al., 2002; Pepovich, 2015); bronchial samples (Sorensen et al., 1997; Caron et al., 2000; Kurth et al., 2002); tracheal samples (Ruiz et al., 2002); tonsillar samples (Kurth et al., 2002); broncho-alveolar fluid (Kurth et al., 2002); pulmonary tissue homogenates (Caron et al., 2000; Rautiainen & Wallgren, 2001); lung tissue (Hipple et al., 2002; Pepovich, 2015) and filtered air from infected barns (Fano et al., 2003).

It should be noted that collecting some of the mentioned samples is a complicated procedure. It's difficult enough when collecting samples from living animals. Additionally, the detection of *M. hyopneumoniae* DNA in nasal secretions via nPCR, which are obtained relatively easy from live and dead pigs, does not confirm the mycoplasma disease because it's not know whether the bacteria was viable or not. The obtained results must be carefully interpreted because the question arises whether those animals are infected or „removing" dead bacteria (Calsamiglia et al., 1999). In experimentally infected pigs, Kurth et al. (2002) test different samples (nasal, tracheobronchal, tonsillar, broncho-alveolar fluid) for proving *M. hyopneumoniae*. The positive samples for discovering the pathogen via nPCR in the nose and tonsils are very low, respectively 15.3% and 0%, compared to 83.3% in bronchial secretions. According to them the broncho-alveolar fluid is best suited for proving *M. hyopneumoniae,* due to the sufficient amount of microorganisms in this sample. They can be discovered with a single PCR and the use of nPCR is not required. In partial disagreement with these results comes the research of Sibila (2004), which establishes that detection of *M. hyopneumoniae* in nasal swabs is a good indicator of its presence in bronchi, the main site of action of the pathogen. Although both upper respiratory locations (nasal and tonsil) were substantially correlated with *M. hyopneumoniae* detection in bronchi, the microorganism was more readily detected in nasal than in tonsillar samples. These results and the fact that nasal swabbing is easier than tonsil sampling, indicate that nasal swabs are the more suitable of the two upper respiratory sites tested for *M. hyopneumoniae* detection in live animals. The discovery of *M. hyopneumoniae* in nasal samples can be useful for establishing heavy infections when the macroscopic lesions are already present. The reason for these differences in the detection of *M. hyopneumoniae* in the upper respiratory tract could be the way of infection, the difference in nPCR, the number of sampled animals and the origin of the samples. On the other hand, the colonization of the ciliary epithelium with micoplasms is described only in the lower compartments of the respiratory tract, which is confirmed by the fact that the cultivation of a culture isolated from nasal secretions is extremely difficult (Mattson et al., 1995; Sorensen et al., 1997).

In recent years, it has been possible to carry out quantitative PCR with high specificity and sensitivity. This method makes it possible to calculate the amount (number) of bacteria in the test sample (Minion et al., 2002).

Serological (immunological) methods

The serological methods are widely used in the diagnosis of enzootic pneumonia in pigs as an assessment of the epizootiological status of the swine population in the farm. Of the serological methods, the enzyme-linked immunosorbent assay (ELISA) is most commonly used for detection of specific antibodies, based on its advantages over other tests, namely: a highly sensitive method; automated testing of a large number of samples for herd diagnostic purposes; detection of all classes of immunoglobulins; gives quantifiable results; a relatively inexpensive method (Calsamiglia et al., 1999; Pepovich, 2015).

According to Maes et al. (1996) the time for detection of antibodies in the blood serum (seroconversion) depends on the infectious pressure and the disease management measures, administered at the farm. Under natural conditions, the time of seroconversion is delayed. It is found after 6-9 weeks from the infection, mainly in weaned and fattening pigs' ages 8 to 24 weeks (Djordjevic et al., 1994; Sitjar et al., 1996; Vicca et al., 2002; Vicca, 2005). In the clinical and subclinical course of EP differences are observed in the time of seroconversion. In clinically ill pigs it is found between 12 – 15 weeks of age, whereas in subclinical herds it is found between 15 – 18 weeks of age. This suggests some delay in the detection time of specific antibodies in a subclinical course of the disease in the pig herd (Vicca et al., 2002).

In the first developed ELISA test for establishing specific antibodies against *M. hyopneumoniae* an unpurified antigen was used (Ross*,* 1999). However, this indirect ELISA gives cross-reactions with *M. floccularae* and *M. hyorhinis*. In order to achieve a higher specificity of the reaction and to avoid cross-serological reactions with other mycoplasma species, Feld et al. (1992) develop blocking ELISA with monoclonal antibodies against *M. hyopneumoniae* 74 kDa protein. Other variants of blocking ELISA with monoclonal antibodies against 40 kDa and 43 kDa protein of *M. hyopneumoniae* were further developed (Le Potier et al., 1994; Djorjevic et al., 1994).

Non-invasive methods for the detection of antibodies in colostrum samples, which may contain immunoglobulins – IgA, IgG and IgM, have also been developed. Such test systems have been used to monitor the epizootiological situation in the reproductive herds (Levonen, 1994; Rautiainen et al., 2000). The main flaw of these methods is that colostrum should be taken immediately after birth, which requires constant monitoring of the sows, but the positive results have a higher degree of reliability than the blood serum test (Stark, 2000; Leon et al., 2001).

Conclusion

The confirmation of *M. hyopneumoniae* infection in pigs remains a challenge for the veterinary practice and science. Determining the involvement of *M. hyopneumoniae* in porcine respiratory diseases can be disappointing, as its establishment is not always linked to the development of a disease process. Diagnosis of enzootic pneumonia requires the application of a complex approach, i.e. the combination of clinical symptoms and lung lesions with the results of various laboratory analyzes. Depending on the stage of the disease and the type of the tested samples, different laboratory tests are used which are characterized by their sensitivity and specificity, on the basis of which an accurate diagnosis can be made.

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