# *Haemophilus parasuis* and Glässer's disease in pigs: a review

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**ABSTRACT**: *Haemophilus parasuis* is a common epiphyte of the upper respiratory tract of pigs. The factors of *H. parasuis* pathogenicity that enable some strains to be virulent and consequently cause a clinical disease have not been established yet. Fifteen serovars of *H. parasuis* have been described at present. Individual serovars differ in virulence, and considerable differences in virulence also exist within each serovar. Virulent strains can particularly participate as microorganisms secondary to pneumonia, cause septicaemia without polyserositis or Glässer's disease characterized by polyserositis, pericarditis, arthritis and meningitis. Clinical symptoms of this disease are highly variable. Therefore, culture detection of causative agent, particularly from the brain, joints and polyserositis is an essential diagnostic tool. The disease caused by *H. parasuis* can be treated with antibiotics; however, oral or parenteral administration of very high doses of antibiotics is necessary. The level of animal hygiene and animal husbandry are important factors for prevention of this disease. Commercial or autogenous vaccines can be used in the immunoprophylaxis of pre-parturient sows and their progeny after weaning. For the production of autogenous vaccines, it is most effective to use isolates from animals with lesions present in CNS. Isolates recovered from arthritic and systemic sites of infection are less suitable and isolates recovered from lungs are not suitable at all because of their heterogeneity.

**Keywords**: *Haemophilus parasuis*; Glässer's disease; diagnosis; therapy and prophylaxis; epidemiology; pig

**Contents**



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## 1. Introduction

Infections caused by *Haemophilus parasuis* in pigs have become worldwide at present. The consequences of these infections are economic losses due to expensive antibiotic treatment and mortality of animals in acute form of the disease. The course of *H. parasuis* infection is particularly serious in specific-pathogen-free (SPF) herds and herds with a good health status where outbreaks are accompanied by high morbidity and mortality. *H. parasuis* is involved in the respiratory syndrome development in conventional herds. Acute infections are occasional and the clinical disease particularly affects young animals exposed to stress. *H. parasuis* can cause Glässer's disease characterized by fibrinous polyserositis, polyarthritis and meningitis (Amano et al., 1994) or acute pneumonia without polyserositis (Little, 1970) and acute septicaemia (Peet et al., 1983). *H. parasuis* endotoxin causes disseminated intravascular coagulation which results in microthrombus formation in various tissues (Peet et al., 1983; Amano et al., 1994). *H. parasuis* is commonly isolated from nasal cavities (Amano et al., 1994; Vahle et al., 1997), tonsils (Oliveira et al., 2001a) and the upper part of trachea (Segales et al., 1997).

The purpose of the present review is to summarize existing knowledge concerning characteristics and diagnosis of *H. parasuis*, to describe development and course of the disease caused by this aetiological agent and to indicate potential treatments, prophylaxis and control of this disease.

## 2. Aetiology of *Haemophilus parasuis*

Gram-negative bacteria from serous exudates of pigs affected by serofibrinous pleuritis, pericarditis, peritonitis, arthritis and meningitis were described for the first time by Glässer (1910). However, this organism was likely isolated for the first time by Schermer and Ehrlich in 1922 (Little, 1970).

According to the original biochemical characteristics, *Haemophilus suis*, which required both X (iron porphyrin), and V (nicotinamide adenine dinucleotide = NAD) growth factors (Lewis and Shope, 1931), was early considered as aetiological agent of Glässer disease. However, Biberstein and White (1969) demonstrated that aetiological agent of Glässer disease was NAD dependent only. Based on the accepted nomenclature of *Haemophilus* genus that uses the prefix "para" for microorganisms not requiring X-factor supplementation, a novel species *H. parasuis* was suggested (Bieberstein and White, 1969).

### 2.1. Morphology of *Haemophilus parasuis*

*H. parasuis* is a gram-negative, non-mobile, small pleomorphic bacterium of *Haemophilus* genus of Pasteurellaceae family (Biberstain and White, 1969). Non-encapsulated strains form various structures from rods to fibres. In some *H. parasuis* strains Morozumi and Nicolet (1986a) detected capsular matter formed by various polysaccharidic structures using heat extraction, separation by electrophoresis and precipitation in Cetavlone (hexadecyl trimethylammonium bromide). Encapsulated strains of *H. parasuis* resemble coccobacilli under microscope, but they can form filaments and fimbriae-like structures, if cultured on chorio-alantoid membrane of chicken embryos (Munch et al., 1992).

### 2.2. Cultivation and biochemical characteristics

Various V-growth factor enriched media are used for the culture (chocolate agar, Levinthal agar, PPLO agar supplemented with NAD) (Nicolet, 1992). *H. parasuis* grows on blood agar in the zone around *Staphylococcus aureus*, does not cause haemolysis, is urease-negative, oxidase-negative, catalase-positive, reduces nitrates, does not produce indol, and causes fermentation of glucose, galactose, mannose, fructose, saccharose and maltose (Kielstein et al., 2001).

It is also possible to obtain other NAD-dependent, non-haemolytic and urease-negative isolates from the respiratory tract of pigs. These isolates were previously determined as *Haemophilus* taxon "minor group" with distinct taxa, which are provisionally designated as taxa C, D, E and F. However, those can be distinguished from *H. parasuis* on the basis of a number of detailed biochemical analyses. Taxa D, E and F constitute common microflora of the upper respiratory tract (Moller and Kilian, 1990), but they can also be isolated from pulmonary tissue (Rapp-Gabrielson and Gabrielson, 1992; Moller et al., 1993) or brain (Rapp-Gabrielson and Gabrielson, 1992; Blackall et al., 1994). Moller et

al. (1993) on the bases of DNA homology studies indicated that taxa D and E belong to one species and these two taxa were combinated. In following study, Moller et al. (1996) proposed three new species, corresponding to the "minor group", taxa D plus E, and taxon F. The names of these new species are *Actinobacillus minor*, *Actinobacillus porcinus* and *Actinobacillus indolicus*, respectively. Comparison of 16S rRNA sequences of all V-factor-dependent bacteria from respiratory tract showed that *H. parasuis* was most related to *A. indolicus* (taxon F), with the degree of similarity ranging from 97.4 to 97.7% (Moller et al., 1996). Minor differences were found between these two species. They consist in the fact that *A. indolicus* can produce acid-derived indol and ferment raffinose (Kielstein et al., 2001).

### 2.3. Serovars of *Haemophilus parasuis*

Studies based on serotyping demonstrated that a high antigenic heterogeneity exists among *H. parasuis* strains. Based on a precipitation test, Bakos et al. (1952) described existence of four *H. parasuis* serovars designated A–D. Later Morozumi and Nicolet (1986b) defined 7 serovars (1–7), Kielstein et al. (1991) added other 6 serovars (Jena 6–Jena 12) and Kielstein and Rapp-Gabrielson (1992) identified additional 5 serovars (ND1–ND5). On the basis of an immunodifusion test (ID) with specific rabbit antisera Kielstein and Rapp-Gabrielson (1992) suggested a novel classification of *H. parasuis* serovars. Classification of the previously defined serovars 1–7 was maintained. The serovars Jena and ND were (after unification of serovars) designated as 8–15. According to the currently worldwide accepted classification, 15 serovars of *H. parasuis* (1–15) have been defined. However, it is necessary to say that a large number of nontypeable *H. parasuis* isolates exist (Kielstein and Rapp-Gabrielson, 1992).

#### 2.3.1. Prevalence of serovars

Several studies for the detection of serovar profiles have been developed in a number of countries worldwide. In Japan (Morikoshi et al., 1990), Germany (Kielstein and Rapp-Gabrielson, 1992), the USA (Rapp-Gabrielson and Gabrielson, 1992), Spain (Rubies et al., 1999), Canada (Tadjine et al., 2004) and China (Cai et al, 2005), serovar 4 was found to be dominant and serovar 5 highly frequent among the *H. parasuis* isolates. Serovars 5 and 13 prevailed among the isolates in Australia (Blackall et al., 1996, 1997; Rafiee and Blackall, 2000) and Denmark (Angen et al. 2004).

### 2.4. Virulence and virulence factors

Virulence factors of *H. parasuis* have not been unequivocally defined yet. Classification of microorganisms to a particular serovar group is usually considered as an indicator of virulence. Intraperitoneal infections with serovars 1, 5, 10, 12, 13 and 14 caused high morbidity or mortality in SPF pigs within 4 days. Accordingly, these strains were considered as highly virulent. Serovars 2, 4 and 15 caused polyserositis without mortality and were designated as intermediately virulent. The remaining serovars (3, 6, 7, 8, 9 and 11) did not cause any clinical signs and are considered as avirulent (Kielstein and Rapp-Gabrielson, 1992; Amano et al., 1994). Serotyping of isolates from clinical cases showed that approximately the same numbers of serovars 2, 4, 5, 12, 13 and 14 were recovered from the respiratory and systemic sites (Rapp-Gabrielson and Gabrielson, 1992; Blackall et al., 1996). The investigation of prevalence of serovars found in North America showed that potentially pathogenic isolates from systemic sites were of serovars 1, 2, 4, 5, 12, 13 and 14 or they were nontypeable. Serovar 3 and nontypeable isolates prevailed in the upper respiratory tract of healthy animals (Oliveira et al., 2003).

Other important virulence factors of the members of family *Pasteurellaceae* that colonize the upper respiratory tract include: capsula, fimbriae, lipopolysaccharides (LPS) and outer membrane proteins (OMP) (Biberstein, 1990). However, the association between expression of these factors and *H. parasuis* virulence is questionable.

Some authors investigated potential association between presence of the capsula and *H. parasuis* virulence using experimental infections. Little and Harding (1971) and Morozumi and Nicolet (1986a) showed, that encapsulated strains particularly occurred among the isolates from nasal cavities of healthy pigs and among the isolates from pathological material, non-encapsulated strains were more frequently. Munch et al. (1992) demonstrated that *H. parasuis* was able to form fimbriae-like struc-

tures after *in vivo* passage. However, their role in virulence has not been clarified.

Another significant virulence factor may be LPS. However, Zucker et al. (1996) did not detect significant differences in the LPS production between virulent and avirulent strains of *H. parasuis.* Similarly, Miniats et al. (1991b) showed that animals vaccinated with bacterin containing LPS and OMP antigens were protected against challenge by the presence of OMP antibodies only. These facts indicate, that LPS are not an important virulence factor in *H. parasuis*. The role of LPS was later investigated by Amano et al. (1997) who found that presence of antibodies against LPS in circulating blood of animals inoculated with *H. parasuis* isolate of serovar 5 was associated with thrombosis and disseminated intravascular coagulation. Furthermore, it is well known that LPS from *H. parasuis* exert endotoxin-like activity, similarly as other gram-negative bacteria do (Raetz and Whitfield, 2002).

Two different OMP profiles, biotype I and biotype II, were identified in *H. parasuis* strains using SDS-PAGE (Nicolet et al., 1980; Morozumi and Nicolet, 1986a; Ruiz et al., 2001). Isolates from nasal mucosa of healthy pigs represent biotype I with proteins of molecular weight of about 68 kDa and between 23–40 kDa. *H. parasuis* isolates from herds affected by Glässer disease were usually of biotype II, which is characterized by a dominant protein with molecular weight of about 37 kDa. These results were later confirmed by computer-based analysis of whole-cell protein profiles of *H. parasuis* (Oliveira and Pijoan, 2004a).

Another potential virulence factor of *H. parasuis* is neuraminidase. Lichtensteiger and Vimr (1997) found production of neuraminidase (sialidase) in more than 90% of field isolates of *H. parasuis*. This enzyme begins to be expressed at the end of the logarithmic phase of the microorganism growth. Receptors necessary for colonization or invasion of host cells can be revealed by the activity of neuraminidase. Defence system of the host can be also affected by decreased viscosity of mucin (Corfield, 1990; Lichtensteiger and Vimr, 1997).

Production of specific toxins for *H. parasuis*, which may be involved in virulence, has not been described yet. It was excluded that *H. parasuis* comprise genes for production of toxins related to *Actinobacillus pleuropneumoniae*, RTX (Apx) toxins (Schaller et al., 2000).

Blackall et al. (1997) attempted to detect differences among *H. parasuis* isolates from systemic and respiratory sites using multilocus enzyme electrophoresis (MEE). They revealed great differences among the isolates and, moreover, they also found great differences among isolates of the same serovar. Two main MEE groups were defined, but the relationship between the site of isolation and MEE profile was not found.

Hill et al. (2003) investigated virulence of the strain of *H. parasuis* 1185 (serovar 5) using differential display RT-PCR. Expression of seven genes was identified in a culture grown at 40°C to mimic conditions during acute disease. These genes were homologous with *fadD* (fatty acyl-CoA synthetase), *apaH* (diadenosine tetraphosphate), *pstI* (enzyme I of the phosphotransferase system), *cysK* (cysteine synthetase), StD ($Na^+$- and $Cl^-$-dependent ion transporter), HSPG (a mammalian basement membrane-specific heparin sulphate core protein precursor) and PntB (pyridine nucleotide transhydrogenase). Expression of the same gene fragments was detected in all 15 serovars of *H. parasuis.* Further detailed analyses are thus clearly necessary for the assessment of virulence factors in *H. parasuis*.

### 2.5. Molecular typing

At present, genotyping methods are used for classification of *H. parasuis* isolates; they allow their better characterization when compared with serotyping. According to the discrimination of isolates on the basis of DNA profiles it has been suggested that certain DNA profiles may be associated with virulence. Isolates obtained from systemic infections form relatively homogenous group in contrast to non-pathogenic strains (Oliveira et al., 2003).

Smart et al. (1988) applied the restriction endonuclease fingerprinting (REF) analysis for investigation of occurrence and distribution of *H. parasuis* isolates in SPF and conventional herds. In majority of SPF herds, identical *H. parasuis* isolates were found; however, high numbers of various isolates were present in conventional herds, and only one of them was shared by all of the examined herds. The profiles of isolates recovered from systemic sites of pigs from herds with enzootic infection were similar, but they differed from the isolates recovered from nasal cavities of healthy pigs from the same population.

*H. parasuis* genotyping by the repetitive element based-PCR (rep-PCR) method was also described (Versalovic et al., 1991, 1994; Woods et al., 1993). This PCR allows amplification of DNA fragments of different sizes by means of ERIC (enterobacterial repetitive intergenic consensus) primers, and reveals specific genome profiles after subsequent separation by electrophoresis (Versalovic et al., 1991; Rafiee et al., 2000; Oliveira et al., 2003). Different DNA profiles were detected by this method even in the isolates of identical *H. parasuis* serovar. A small number of isolates with similar DNA profiles were responsible for mortality in the affected herds (Oliveira et al., 2003).

Another alternative method of *H. parasuis* typing is the PCR – restriction fragment length polymorphism (RFLP) test. By typing the *H. parasuis* isolates using the PCR-RFLP analysis of *tbpA*, a gene that encodes transferrin-binding protein, 12 different profiles for reference strains of 15 serovars of *H. parasuis* were found. Reference strains of serovars 5, 12, 14 and 15 were of identical restriction profiles. Thirty-three RFLP profiles were identified during characterization of 101 field isolates of *H. parasuis;* 10 of them were identical with profiles of reference strains. No correlation of serovar and RFLP type was found (Redondo et al., 2003).

## 3. Pathogenesis of *Haemophilus parasuis* infections

Initial site of *H. parasuis* colonization of the upper respiratory tract of pigs has not been identified yet. Vahle et al. (1995) intranasally infected caesarean-derived colostrum-deprived (CDCD) piglets at the age of 5 weeks. *H. parasuis* was isolated 36 h post-inoculation from blood, nasal cavities and trachea, less frequently from lungs and blood smears; however, it was not isolated from tonsils (Vahle et al., 1997). Amano et al. (1994) successfully isolated *H. parasuis* from nasal cavities and tonsils after intranasal inoculation of pigs with serovars 1, 4 and 5. Segales et al. (1997) described common isolation of *H. parasuis* from tonsil and tracheal swabs from pigs after intratracheal inoculation and Kirkwood et al. (2001) isolated *H. parasuis* from nasal cavity swabs from infected pigs.

Factors involved in the *H. parasuis* invasion into systems during infection have not been thoroughly recognized yet. Vahle et al. (1997) demonstrated that isolation of *H. parasuis* from the middle part of nasal cavity was accompanied with acute suppurative rhinitis and loss of mucocilliary cells. The authors also suggested that these mucosal alterations might facilitate invasion of *H. parasuis* and their access to the blood circulation. However, it was not possible to detect *H. parasuis* in the sites of lost cilia and mucosal cell degeneration either by electron microscopy or immunohistochemistry.

Brockmeier (2004) demonstrated that *Bordetella bronchiseptica* was involved as a predisposing factor for *H. parasuis* colonization of the upper respiratory tract, similarly as in the infections caused by *Pasteurella multocida* in atrophic rhinitis of pigs.

## 4. Epidemiology of the disease

The course of *H. parasuis* infection is enzootic. The disease is most commonly transmitted by direct contact; indirect transmission is only hypothetical. Pathogenic strains are introduced into a herd by purchase of animals from infected herd. All age categories are susceptible to the infection and may be involved in an outbreak of the disease. Similar situation can occur in an infected herd when a new, antigenically different, virulent strain is introduced (Oliveira and Pijoan, 2002).

Sows are reservoirs of the disease in infected herds. Piglets are colonized with *H. parasuis* during whole period of suckling since the sows shed both pathogenic and non-pathogenic strains during this period. However, frequency of shedding of *H. parasuis* by the sows is very low; accordingly, only a small proportion of piglets are colonized. These piglets consequently develop own immunity and later become subclinical carriers. The other piglets that are not colonized with pathogenic strains are protected by colostral immunity in this period. The level of colostral antibodies decreases after weaning, at the age of 5–6 weeks. Frequency of shedding of pathogenic strains by subclinical carriers increases due to the post-weaning stress and the disease affects animals that have not been colonized with pathogenic strains during suckling. These animals are not protected by either colostral immunity or their own post-infection immunity. Accordingly, they are highly susceptible to the infection. Therefore, this disease is usually clinically manifested after weaning at the age of 5–6 weeks (Solano-Aguilar et al., 1999; Oliveira and Pijoan, 2002).

## 5. Clinical symptoms of *Haemophilus parasuis* infections

Clinical symptoms depend on the affected body site of the animal. Hoefling (1994) described four forms of the *H. parasuis* infection: Glässer's disease (fibrinous polyserositis), septicaemia (without polyserositis), myositis acuta (masseter muscle) and respiratory disease.

Clinical signs of all forms of the disease caused by *H. parasuis* are mostly non-specific. The course of the disease is peracute or acute and animals with a high health status usually develop the disease. The first clinical signs are increased body temperature, apathy and inappetence. The following signs can also be observed in the affected animals: cough, dyspnoea, body weight loss, lameness, incoordination, flare or cyanosis, decubitus; some animals may die of exhaustion (Rapp-Gabrielson, 1999). The other acute bacterial and viral diseases with concurrent fever caused e.g. by *Actinobacillus pleuropneumoniae*, *Actinobacillus suis*, *Streptococcus suis*, *Erysipelothrix rhusiopathiae*, *Mycoplasma hyorhinis*, *Influenzavirus* are necessary to exclude in terms of differential diagnosis (Nicolet, 1992; Rapp-Gabrielson, 1999).

## 6. Methods of diagnosis

Significance of diagnosis based on clinical signs is low due to their non-specificity and further examinations are therefore necessary.

### 6.1. Pathological lesions

Primary finding during post-mortem examination is serofibrinous or fibrino-purulent exudate on mucosal surface, usually in peritoneum, pericardium, pleura or joint surface. Histopatological findings in fibrinopurulent inflammations are infiltrates of neutrophils and, to lower extent, of macrophages. In more severe cases, meningitis, thrombotic meningoencephalitis, accompanied with increased production of cerebrospinal fluid and arthritis are found. Pneumonic lesions are not usually observed, even in cases with subsequent recovery of *H. parasuis* from the lungs. In case of septicaemia, petechia and ecchymosis are detected in liver, kidneys and brain concurrently with the occurrence of increased levels of endotoxins in blood plasma and fibrin clots in various organs. Acute cases of septicaemia accompanied with signs of cyanosis, subcutaneous and pulmonary oedema are less frequent; typical signs of mucosal inflammation are not found. It is necessary to primarily exclude *S. suis* infection by differential diagnosis in a case of a systemic disease; fibrinous pleuritis may develop also due to other bacterial infections, such as particularly *A. pleuropneumoniae* (Nicolet, 1992; Amano et al., 1994).

### 6.2. Isolation of *Haemophilus parasuis* from clinical samples

*H. parasuis* isolation from clinical samples is performed on blood agar close to *S. aureus* culture as a source of NAD, on chocolate agar or on NAD supplemented PPLO media. Cultivation time between 24–48 h is recommended (Segales et al., 1997). *H. parasuis* is one of demanding microorganisms; its isolation from clinical samples is usually difficult and often complicated by contaminations. That is why application of a diluted mixed culture on agar or culture in media supplemented with antibiotics such as lincomycin and bacitracin are sometimes recommended (Pijoan et al., 1983). It is necessary to consider that *H. parasuis* is a common epiphyte of the upper respiratory tract and its isolation from the respiratory tract does not necessarily confirm a systemic *H. parasuis* infection. Significant for the diagnosis is therefore detection of *H. parasuis* in the brain or joints (Oliveira and Pijoan, 2002). When *H. parasuis*-like microorganisms are successfully isolated, biochemical tests must be performed to differentiate them from the other non-haemolytic NAD-dependent bacteria, such as *Actinobacillus indolicus, Actinobacillus porcinus* and *Actinobacillus minor* (Kielstein et al., 2001; Oliveira et al., 2001b).

### 6.3. Serotyping

As noted above, 15 serovars of *H. parasuis* (1 to 15) have been described on the basis of the ID test with specific rabbit antisera (Kielstein and Rapp-Gabrielson, 1992). Recently, several studies based on comparison of different methods of serological typing of *H. parasuis* strains (ID test, IHA, coagglutination tests) have been published (Del Río et al., 2003; Tadjine et al., 2004; Turni and Blackall, 2005).

Turni and Blackall (2005) compared ID and IHA tests in field isolates. These authors used the correct and validated method for producing antigen to be used in the ID test within the KRG serotyping scheme. They found more nontypeable isolates by the IHA test (44%) compared to the ID test (41%). Some of the studies described that the most suitable method of *H. parasuis* serotyping was IHA; that method decreased the percentage of serologically nontypeable strains (by ID or CA tests) to less than 10% (Del Rio et al., 2003; Tadjine et al., 2004). The studies of Del Rio et al. (2003) and Tadjine et al. (2004) have some deficiencies. Neither study confirmed that the strains used in the study were the accepted international reference strains. As well, Tadjine et al. (2004) used an antigen extraction technique that has never been validated with the GD test. Hence, all conclusions reached in these papers need to be balanced by these deficiencies.

The nontypeable isolates may either exist due to the fact that some isolates do not likely contain a sufficient level of serovar-specific antigens or that there exist novel serovars that have not been identified yet. The difference between the ID and IHA tests consists in the fact that a soluble, naturally precipitating antigen is used for ID. Erythrocytes coated with a soluble antigen are used in the IHA test; consequently, precipitating antigens change into agglutination antigens and sensitivity of the IHA test is up to 3000x higher than that of the immunodiffusion test (Mittal, 2003).

### 6.4. Antibody detection

Complement-fixation test (CF) (Nielsen, 1993; Takahashi et al., 2001), IHA (Miniats et al., 1991a) and ELISA test (Miniats et al., 1991a; Solano-Aguilar et al., 1999) can be used for the detection of antibodies against *H. parasuis*. Circulation of antibodies has been demonstrated in clinical cases with an acute course of the disease for approximately 1 week and considerable cross-serovar reactivity was observed (Nielsen, 1993). Takahashi et al. (2001) performed a vaccination experiment and measured titres of antibodies by the CF test. Positive titres were recorded 19 days after the second vaccination. After IHA, with sonicated or boiled *H. parasuis* cells as antigens used for coating of sheep erythrocytes and the ELISA test, where either supernatants from boiled bacteria or dialyzed hot extract of bacteria in phenol : water as surface antigen were used, variable, largely negative results were obtained, especially in vaccinated animals. Accordingly, these tests are not suitable for the detection of protective immunity against *H. parasuis* (Miniats et al., 1991a). However, ELISA test with formalin-inactivated whole cells can be used for the investigation of titres of antibodies in dams and the immune response of piglets after vaccination (Solano-Aguilar et al., 1999).

### 6.5. Molecular biological methods

One of the potential diagnostic tools is a highly sensitive oligonucleotide-specific capture plate hybridization (OSCPH) (Calsamiglia et al., 1999) that can detect $<10^2$ CFU/ml in a pure culture. Due to the fact that it is not always possible to obtain a pure culture of *H. parasuis*, a PCR test was developed that enables specific identification ($10^2$ CFU/ml) of this microorganism directly from clinical samples, and moreover, it can detect non-living organisms (Oliveira et al., 2001b). However, a weak positive reaction with *A. indolicus* is obtained by both OSCPH and PCR. Therefore, it was recommended to use these tests for examination of isolates recovered from systemic sites only, because *A. indolicus* is common epiphyte of the upper respiratory tract (Oliveira and Pijoan, 2004b).

### 6.6. Immunohistochemical diagnosis

Since *H. parasuis* isolation from clinical samples is difficult due to contamination by less demanding microorganisms, immunohistochemical (IHC) examination is recommended for diagnosis of *H. parasuis* infections. IHC allows detection of non-living microorganisms in the cytoplasm of phagocytes (Amano et al., 1994; Segales et al., 1997). However, some polyclonal antibodies used for diagnosis of *H. parasuis* by the IHC method cross reacted with *A. pleuropneumoniae* (Segales et al., 1997).

## 7. Treatment and prevention of the disease

Poor animal hygiene, inadequate nutrition and herd management are predisposing factors to the development of *H. parasuis* infection, similarly as it is in other infections. Non-supervised transport and rearing animals of different age in the same

stable are factors particularly involved in outbreaks of the disease (Rapp-Gabrielson, 1999).

### 7.1. Antibiotic treatment

The diseases caused by *H. parasuis* can be treated with antibiotics. Parenteral antibiotic therapy should be started as soon as possible after manifestation of clinical signs. Recommended doses of antibiotics differ according to the character of infection. High doses are necessary during outbreak of Glässer disease because of penetration of the causative agent into tissues and cerebrospinal fluids, and diffusion into affected joints (Nicolet, 1992).

Medication should be initiated on the basis of sensitivity determination of isolated strains to respective antibiotics. There are only very few reports on the antimicrobial resistance in *H. parasuis*. All *H. parasuis* isolates in Switzerland were susceptible to penicillin and enrofloxacin, but resistance was observed to streptomycin, kanamycin, gentamicin, tetracycline, erythromycin, sulfonamide and TMP + sulphonamide (Wissing et al., 2001). The Danish isolates of *H. parasuis* were fully susceptible to tested antimicrobial agents (ampicilin, ceftifour, ciprofloxacin, erythromycin, fluorphenicol, penicillin, spectinomycin, tetracycline, tiamulin, tilmicosin and TMP + sulfamethoxazole) by MIC-determinations (Aarestrup et al., 2004). However, these differences among results of these studies could be caused by antibiotic policy used in particular countries, which can have a major effect on the occurrence of resistant isolates.

### 7.2. Immunoprophylaxis and vaccination

Another possibility how to control *H. parasuis* infection is vaccination. Although virulence factors and protective antigens of *H. parasuis* are not known, it is widely accepted that serovar-specific immunity exists. Effective prevention of *H. parasuis* infections can be achieved by the use of commercial (Riising, 1981; Solano-Aguilar et al., 1999; Bak and Riising, 2002; Baumann and Bilkei, 2002) or autogenous vaccines (Smart et al., 1993; Kirkwood et al., 2001). Although identification of the serovar is one of essential criteria in the immunoprophylactic programmes based on autogenous vaccines, it is necessary to consider other factors that are significant for the selection of a vaccination strain. Various isolates ranging from highly virulent to avirulent ones can be obtained from one animal. Accordingly, it is recommended to use isolates recovered from brain for production of autogenous vaccines; isolates recovered from joints and systemic infections are less suitable and isolates from lungs are not suitable at all because of their high heterogeneity (Oliveira and Pijoan, 2002).

Antigenic characteristics of *H. parasuis* were assessed according to the study of immune response to phenotypical markers such as OMP, LPS and capsular polysaccharides. Miniats et al. (1991b) investigated humoral response of vaccinated pigs to these antigens by an immunoblotting test and found that only presence of antibodies to OMP was associated with the protection against challenge. Fully protected animals did not have antibodies against LPS or capsular polysaccharides after vaccination. Moreover, Rapp-Gabrielson et al. (1997) found out that various strains of identical serovar group may differ in capability to protect against homologous challenge, despite of having identical OMP and LPS profiles.

The effort invested in the development of effective vaccines against *H. parasuis* is focused on development of cross-protective immunity, due to variability of serovars and high percentage of non-typeable isolates.

Miniats et al. (1991b) tried to induce cross-protective immune response by means of bacterins containing highly or less virulent strains of *H. parasuis*. Cross-protection against homologous and heterologous challenge was exclusively obtained with virulent strains; pigs vaccinated with bacterin containing strains of low virulence were only protected against homologous challenge.

Rapp-Gabrielson et al. (1997) studied cross-protection after administration of bacterin vaccines among serovars 2, 4, 5, 12, 13 and 14, which were reported as the most prevalent in the US in 1992. After immunization with these bacterins, the protection was found after homologous challenges with all tested serovars, with the exception of bacterin, prepared from serovar 12 and bivalent bacterins prepared from serovars 2 and 12. The cross-protection was found after application of bacterin prepared from serovar 4 against challenge with serovar 5 and bivalent bacterin from serovars 4 and 5 against challenge with serovars 13 and 14 (significantly decreased severity of lesions and mortality of piglets). In this study, other

tests were performed with the aim to investigate whether bacterins prepared from serovar 12 could provide protection against homologous challenge. Comparison of different isolates of serovar 12 (12a and 12b) showed that vaccination with bacterin prepared from isolate 12a did not protect against homologous challenge, whilst vaccination with isolate 12b provided significant protection against homologous challenge. Despite the fact that both isolates of serovar 12 were highly virulent and produced similar OMP and LPS profiles, differences in the expression of protective antigens in bacterin must have been present.

In the study provided by Takahashi et al. (2001), the cross-protection among serovars 2 and 5 was not demonstrated after immunization with monovalent bacterin. However, sufficient protection was found after immunization with bivalent vaccine included serovars 2 and 5 against challenge by lethal dose with each of the serovar strains.

Bak and Riising (2002) studied protection *H. parasuis* vaccine contained serovar 5 cells and Diluvac Forte (Intervet) adjuvans. After vaccination of piglets at the age of 5 and 7 weeks, the protection was found protection not only against homologous challenge with serovar 5, but also was demonstrated clear protection against heterologous challenge with serovars 1, 12, 13 and 14. The results of cross-protection against serovars 13 and 14 are in accordance with study of Rapp-Gabrielson et al. (1997).

Cross-protection was obtained on the basis of mucosal and cell immunity stimulation. Nielsen (1993) intranasally infected SPF pigs with isolates of serovars 1–7. Although only serovars 1 and 5 caused a systemic disease in the inoculated animals, pigs infected with aerosol containing serovars 2, 3, 4 and 7 were also resistant to subsequent challenge with a virulent isolate of serovar 5.

With the aim to prevent mortality in rearing facilities, piglets were exposed to low doses of live, virulent isolates of *H. parasuis*. This method is based on a hypothesis that only few pigs in a herd are naturally colonized with virulent isolates of *H. parasuis.* Early colonized pigs are protected against systemic infections by colostral antibodies. When colostral immunity decreases, pigs are capable of active immune response. After weaning, these piglets are a source of infection for the animals that were not previously colonized with virulent strains present in the herd. These piglets are highly susceptible and developed systemic diseases at the age of 6–8 weeks, i.e. at the period when the level of colostral antibodies does not provide protection any more (Pijoan et al., 1997). A field experiment was performed to test this hypothesis. Piglets at the age of 5 days were orally inoculated with a dose of $7 \times 10^3$ CFU in a suspension containing live virulent isolates of *H. parasuis*. Decreased mortality by 2.88% was recorded in the exposed pigs compared with control group. However, this method is not recommended in herds where sows are affected by PRRSV (Oliveira et al., 2001a).

Another problem arising from the vaccination against *H. parasuis* is the appropriate time of administration. The concern that colostral immunity may adversely affect active immunity formation after vaccination (Bak and Riising, 2002) has been opposed by some other authors (Solano-Aguilar et al. 1999; Baumann and Bilkei, 2002). They demonstrated that vaccination of sows and piglets was effective, and colostral antibodies did not seem to interfere with vaccination. On the other hand, vaccination of piglets was not effective, if sows were not vaccinated either. It is necessary to design the vaccination strategy to ensure immunity stimulation of piglets both before and after weaning. Oliveira and Pijoan (2002) also confirmed, that colostral immunity after vaccination of pre-parturient sows and subsequent vaccination and revaccination of weaned piglets can provide necessary protection.

## 8. Conclusion

Interest of many research teams in *H. parasuis* infections has increased over past years. However, a number of questions have not been answered yet, and further detailed analysis aimed particularly at the detection of virulence factors, mechanisms of their action, improvement of diagnostic methods and development of novel effective wide spectrum vaccines is still necessary.

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