Veterinary Microbiology 242 (2020) 108595

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Review article

Update on Glässer's disease: How to control the disease under restrictive use of antimicrobials

Mar Costa-Hurtado[a], Emili Barba-Vidal[b], Jaime Maldonado[b], Virginia Aragon[a,c,*]

[a] IRTA, Centre de Recerca en Sanitat Animal (CReSA, IRTA-UAB), Campus de la Universitat Autònoma de Barcelona, Bellaterra, 08193, Spain
[b] HIPRA, Av. de la Selva 135, Amer, 17170, Girona, Spain
[c] OIE Collaborating Centre for the Research and Control of Emerging and Re-emerging Swine Diseases in Europe (IRTA-CReSA), Bellaterra, Barcelona, Spain

ARTICLE INFO

Keywords:
Glässer's disease
Glaesserella (*Haemophilus*) *parasuis*
Vaccines
Antimicrobials
Nasal microbiota

ABSTRACT

Antimicrobials have been commonly used to control bacterial diseases in farm animals. The efficacy of these drugs deterred the development of other control measures, such as vaccines, which are currently getting more attention due to the increased concern about antimicrobial resistance. Glässer's disease is caused by *Glaesserella* (*Haemophilus*) *parasuis* and affects pork production around the world. Balance between colonization and immunity seems to be essential in disease control. Reduction in antimicrobial use in veterinary medicine requires the implementation of preventive measures, based on alternative tools such as vaccination and other strategies to guarantee a beneficial microbial colonization of the animals. The present review summarizes and discusses the current knowledge on diagnosis and control of Glässer's disease, including prospects on alternatives to antimicrobials.

1. Introduction

Since the discovery of antimicrobials, bacterial diseases have been commonly controlled by using these drugs. The effectiveness of antimicrobials resulted in the development and use of vaccines against these infections becoming a lesser priority. In veterinary medicine, the lag in the development of efficient vaccines against bacterial diseases is even more striking than in human medicine (main commercial bacterial vaccines used in veterinary medicine are listed in Table 1). However, the increased concern about antimicrobial resistance is promoting the research in efficient vaccines and vaccine use.[1]

Pork represents about 36% of the total meat produced worldwide (FAO meat market report 2018). Pig production is present in all continents, with China, the EU and the USA producing more than 90 million tons of pork in 2018 (FAO meat market report 2018). Health is one of the most important contributors to productivity, profitability and animal welfare in pig production today. Glässer's disease remains a significant economic burden for the pig industry, with major impact in the nursery and early fattening stages. Acute outbreaks of Glässer's disease can cause high mortality (typically between 5–10% in young animals), together with high costs due to productive impairments, carcass disposal and associated treatments (http://www.nadis.org.uk/bulletins/glaessers-disease.aspx). Moreover, *G. parasuis* typically complicates infections by other primary pathogens, such as influenza virus or porcine reproductive and respiratory syndrome (PRRS) virus, worsening the production parameters. Unfortunately, the precise burden of disease, as well as the contribution of different risk factors to Glässer's disease development, have not been fully quantified.

Although the use of antimicrobials continues to be a common means to treat this infection, the economic impact of the disease for pig production can also be inferred by vaccine sales. The market for Glässer's disease licensed monovalent vaccines in Europe was estimated to be of €7M, and over €15M globally on 2018; with a trend to increase in countries with highly restricted use of antimicrobials (HIPRA, unpublished data). In addition, awareness on the emergence of antimicrobial resistance presses for the implementation of alternatives for disease control. Antimicrobial resistance (AMR) is a serious threat to animal and public health, and international policies are demanding a reduction in their use, especially in the farming industry (https://amr-review.org). Although global efforts to reduce the use of antimicrobials in pig farming have been adopted internationally, compensatory strategies for disease control are still needed.

* Corresponding author at: IRTA, Centre de Recerca en Sanitat Animal (CReSA, IRTA-UAB), Campus de la Universitat Autònoma de Barcelona, Bellaterra, 08193, Spain.
E-mail addresses: mar.costa@irta.cat (M. Costa-Hurtado), emili.barba@hipra.com (E. Barba-Vidal), jaime.maldonado@hipra.com (J. Maldonado), virginia.aragon@irta.cat (V. Aragon).

[1] https://www.ema.europa.eu/en/veterinary-regulatory/research-development/availability-veterinary-vaccines.

https://doi.org/10.1016/j.vetmic.2020.108595
Received 17 December 2019; Received in revised form 20 January 2020; Accepted 23 January 2020

Table 1
Main commercial bacterial vaccines used in swine medicine.

	Disease	Bacterial agent
Respiratory	Enzootic pneumonia	*Mycoplasma hyopneumoniae*
	Atrophic rhinitis	*Bordetella Bronchiseptica, Pasteurella multocida D*
	Porcine pleuropneumonia	*Actinobacillus pleuropneumoniae*
	Pasteurellosis	*Pasteurella multocida A + D*
Reproductive	Leptospirosis	*Leptospira* sp.
Digestive	Neonatal diarrhoea	*Escherichia coli (enterotoxigenic) Clostridium perfringens*
	Colibacillosis	*Escherichia coli (enterotoxigenic)*
	Proliferative enteropathy	*Lawsonia intracellularis*
	Salmonellosis	*Salmonella* Typhimurium
Systemic	Gas gangrene, clostridial cellulitis	*Clostridium Novyi*
	Salmonellosis	*Salmonella* Choleraesuis
	Glässer's disease	*Glaesserella (Haemophilus) parasuis*
	Swine erysipelas	*Erysipelothrix rhusiopathiae*
	Edema (Oedema) disease	*Escherichia coli (verotoxigenic)*
	Streptococcal infection	*Streptococcus suis*

2. Pathogenesis of Glässer's disease

Haemophilus parasuis, recently renamed *Glaesserella parasuis* after detailed phylogenetic analysis (Dickerman et al., 2019), is a Gram-negative bacterium of the *Pasteurellaceae* family, which is present in most commercial pig farms as an early colonizer of piglets. In fact, the bacterium can be detected in the nasal cavity of piglets as soon as 2 days after birth (Cerdà-Cuéllar et al., 2010). However, the onset of Glässer's disease occurs later, after weaning, normally linked to situations of stress along with a decrease in maternal immunity (Aragon et al., 2019).

2.1. Diversity of G. parasuis strains and pathogenesis of disease

G. parasuis is a heterogeneous bacterial species that comprises strains with wide differences in virulence (Kielstein and Rapp-Gabrielson, 1992; Aragon et al., 2010). Non-virulent strains are considered part of the normal nasal microbiota of piglets, while virulent strains are considered primary pathogens and the cause of Glässer's disease (Aragon et al., 2019). This duality needs to be considered when diagnosing and controlling the disease. Classically, *G. parasuis* strains have been classified into 15 serovars, some of them associated to virulence (Kielstein and Rapp-Gabrielson, 1992). Serovars 4 and 5 are the most commonly reported from clinical cases, but serovars 2, 12, 13, 14 and 15 are also frequently isolated (Castilla et al., 2012; Zhang et al., 2012).

Comparisons between virulent and non-virulent strains of *G. parasuis* have allowed the description of some mechanisms of pathogenesis of this bacterium. *G. parasuis* is able to colonize mucosae by adhering to the mucus layer and the underlying epithelium (Bouchet et al., 2009; Frandoloso et al., 2012; Bello-Orti et al., 2014). Virulent and non-virulent strains can be detected in the upper respiratory tract, but differences in colonization capacity are revealed in their progression to the lower respiratory tract. Once in the trachea, virulent strains demonstrate higher colonization capacity (Bello-Orti et al., 2014). *G. parasuis* virulence is associated to evasion of the innate immune system by degradation of IgA (Mullins et al., 2011), resistance to phagocytosis by alveolar macrophages (Olvera et al., 2009) and resistance to serum complement (Cerdà-Cuéllar and Aragon, 2008). Thus, virulent strains survive in the lung, while non-virulent strains are cleared by the action of phagocytes, mainly alveolar macrophages (Olvera et al., 2009; Bello-Orti et al., 2014). Virulent *G. parasuis* can subsequently spread systemically, causing a strong inflammation, which together with the disruption of adherens junctions and increased vascular permeability, result in considerable fibrin exudates (Costa-Hurtado et al., 2013; Hua et al., 2018). These processes can explain the characteristic findings of fibrinous polyserositis observed in Glässer's disease.

2.2. Factors affecting disease onset

Although *G. parasuis* is endemic in most pig farms, Glässer's disease is only observed in some cases. Some risk factors affecting disease development, common to other diseases in swine, are the environmental conditions of the facilities, stress, management practices and concurrent presence of other pathogens (Pereira et al., 2017). Exacerbation of clinical signs and lesions by co-infections with *G. parasuis* and viruses, such as swine influenza virus or/and PRRS virus are well known by clinical practitioners, but also supported by experimental data showing stronger deleterious inflammatory responses in co-infections (Li et al., 2017b; Pomorska-Mol et al., 2017). These viral infections disturb the pig immune response, increasing the likelihood of developing disease, even by *G. parasuis* strains with low pathogenic potential (Olvera et al., 2009).

Specific risk factors for Glässer's disease include the immunity against *G. parasuis*, which will be discussed later, and the virulence of the strains circulating in the farm. In fact, the presence of virulent strains in the nasal cavity of pigs constitutes a risk factor for the development of disease, while colonization by non-virulent strains may confer protection against subsequent exposure to virulent strains, and therefore against disease (Brockmeier et al., 2013). Colonization by *G. parasuis* can also be driven by other factors, such as medication, vaccination and sow parity. Moreover, the global composition of the nasal microbiota at weaning has been found to influence the later development of Glässer's disease (Correa-Fiz et al., 2016). As also reported for other maladies, a lower diversity in the nasal microbiota at weaning was associated with higher risk of suffering Glässer's disease in the nursery (Correa-Fiz et al., 2016). Taking this observation into account, factors negatively affecting bacterial diversity, such as the use of preventive antimicrobials, may have the opposite effect, triggering the development of disease.

3. Diagnosis

3.1. Clinical and pathological diagnosis

Diagnosis of Glässer's disease starts at the farm with the observation of clinical signs, followed by a pathological diagnosis. Clinical signs of Glässer's disease are non-specific and include fever, cough, abdominal breathing, swollen joints and lameness, as well as signs of central nervous system impairment such as lateral decubitus, pedaling and tremors (Aragon et al., 2019). Piglets with acute disease show characteristic lesions of fibrinous polyserositis, and occasionally purulent catarrhal pneumonia, at postmortem examination (Aragon et al., 2019). Chronically affected animals may show reduced growth and fibrinous polyserositis lesions at necropsy (Aragon et al., 2019). Clinical and pathological observations need to be always complemented with laboratory confirmation of the aetiological agent, to rule out other pathogens that can cause similar gross lesions, such as *Streptococcus suis* or *Mycoplasma hyorhinis* (Aragon et al., 2019).

3.2. Laboratory diagnosis

Detection of the pathogen is commonly achieved by bacterial isolation and molecular detection by PCR (Angen et al., 2007). Although PCR speeds up diagnosis, bacterial isolation is recommended when supplemental characterization of the strains is necessary, such as antimicrobial susceptibility. As mentioned previously, not all the *G. parasuis* strains have the same ability to cause disease. Characterization of the pathogenic potential of an isolate is a complex process that involves *in vitro* and *in vivo* virulence tests. While this obviously cannot be done in routine diagnosis, this information has been important for the design of

molecular tests, together with analysis of the strains' genomes. It should be noted that PCR tests are now available to determine the serovar and pathogenic capacity of strains of *G. parasuis* (see below).

3.3. Samples for diagnosis

The culture of *G. parasuis* in the laboratory is not always successful. It is convenient to collect samples from more than one animal with clear symptoms of the disease and not treated with antibiotics. If the lesions are systemic, the sampling should include all those organs that may be involved in the symptoms, such as the brain, joints and serous surfaces. A high number of samples increases the possibility of detecting this fastidious bacterium. Samples from the respiratory tract are problematic, since colonizing strains, which may include non-virulent strains, could be aspirated to the lung during euthanasia. Thus, lung samples should only be taken in the event of pneumonia and from distal parts (lungs and distal bronchioles). It is also crucial to prevent contamination of the samples by using an aseptic method and ensure a fast transport to the laboratory under refrigeration (4 − 8 °C). Particularly, in the case of swabs, Amies transport medium is recommended to preserve viability of the bacteria (del Rio et al., 2003).

3.4. Antimicrobial susceptibility testing

Antimicrobial therapy needs to be preceded by determination of the antimicrobial susceptibility of the clinical isolate. Standard methods and specific breakpoints for *G. parasuis* have not been established yet. Recently, some efforts have been made in the harmonization of the broth microdilution susceptibility test (Brogden et al., 2018; Prüller et al., 2016). Information on the minimal inhibitory concentration (MIC) values will result in a more educated decision for treatment.

3.5. PCR associated with virulence and molecular serotyping

The situation in the farm is usually complex, where is common to find diverse strains infecting concurrently the same population of pigs (Cerdà-Cuéllar et al., 2010). Isolation of bacteria from systemic lesions is an indication of the virulence of the strain, as the capacity to penetrate systemically has been described as a virulence trait. However, this cannot be assumed for samples in the respiratory tract, because *G. parasuis* has been isolated in the slaughterhouse on bronchi (3.5% prevalence) and lungs (2% prevalence) from animals without clinical signs of pneumonia (Palzer et al., 2008).

Serovar determination can be useful for vaccine selection, and molecular serotyping has become a major advance in *G. parasuis* diagnosis (Howell et al., 2015). Molecular serotyping is easy to standardize and implement and has been able to significantly reduce the number of non-typeable strains reported by immunological methods. However, the congruency of results with antigenic serotyping is not total and further validation with more strains will be useful (Ma et al., 2016).

The possibility of differentiating virulent from non-virulent strains has improved radically the diagnosis of the disease. Genomic analyses have generated information for the design of simple molecular tests for identification of strains with higher virulence potential. Several genes have been proposed as virulence markers and tested with reference and field strains (Galofre-Mila et al., 2017; Turni et al., 2018). Recently the leader sequences of the virulence-associated trimeric autotransporter (*vtaA*) genes have been used to predict the virulence of strains by PCR (Galofre-Mila et al., 2017). Although the association of this PCR with virulence was demonstrated, further analysis with more field strains is required for complete validation of the technique (Turni et al., 2018). In addition, in agreement with the multifaceted nature of the pathogenicity of *G. parasuis*, a more complex PCR has been developed to detect a set of genes whose presence was associated with the clinical origin of the strain (Howell et al., 2017). These PCRs designed to detect virulent strains could be used in nasal samples to assess carriers of virulent strains and evaluate the risk of developing Glässer's disease on each farm.

4. Control

Three key elements in the control Glässer's disease are herd management, use of antimicrobials and immunization by vaccination. Since sows are the only known source of the bacterium, they need to be included in control programs.

4.1. Herd management

Some stressful practices such as early weaning, transportation, inadequate temperatures or air draught, among others, have been described to cause immunosuppression and trigger the disease onset if virulent bacteria are present (Pereira et al., 2017). Hence, efforts should be done to achieve management practices that minimize stressful situations, particularly on the weaning phase.

4.2. Antimicrobial therapy

Antimicrobials are extensively used for the control of bacterial diseases in pigs, including Glässer's disease. However, such use is closely related to an increase of antimicrobial resistance, which is currently one of the world's biggest concerns in terms of animal and public health. Mitigation of this problem relies on a prudent use of antimicrobials, while effective alternatives to combat bacterial diseases, especially for veterinary medicine, are scarce. The need to reduce the use of antimicrobials is central to many health policies in both human and veterinary medicine.

The world organization for animal health (OIE) standards provide global recommendations for controlling antimicrobial resistance, including lists of antimicrobial agents of veterinary importance to treat animal diseases. In parallel, the world health organization (WHO) has also developed a list of critically important antimicrobial agents in human medicine. The overlap of critical lists for human and veterinary medicine leads to guidelines, such as the European 2015/C 299/04, for more restricted antimicrobial interventions for a sustainable pig production.

When antimicrobials are used to treat Glässer's disease, treatment should start as soon as the first clinical signs are observed, since usually, pigs treated early during infection are able to recover. However, field data indicate that antimicrobial treatment can select resistant strains of *G. parasuis*, and can lead to drug and multidrug resistance in *G. parasuis* isolates (Table 2 and Fig. 1). It is particularly concerning that, for example, two out of three antibiotics for which resistance was recently detected in more than 50% of the Spanish isolates (flumequine and neomycin; Fig. 1) are classified on the Spanish national AMR surveillance plan (PRAN) as category 2 (2nd choice or last resort use in veterinary medicine) and categorized by the WHO as Critically Important of priority 1 and 2.

Antimicrobial resistance has been also detected in nasal isolates from healthy pigs (Moleres et al., 2015; Zhang et al., 2019). This finding highlights the importance of colonizing strains as reservoir of drug resistance; even more if we consider that some resistance genes in *G. parasuis* could be mobilized by plasmids (Li et al., 2015; Moleres et al., 2015; Zhang et al., 2018).

Antimicrobials can also hinder the development of an effective protective immune response against future infections by virulent *G. parasuis* (Macedo et al., 2017). The use of antimicrobials around the time of colonization of the upper respiratory tract can interfere with the colonization by *G. parasuis*, and importantly with the onset of immunity and posterior protection of the piglets (Macedo et al., 2017). This interference is not limited to *G. parasuis*, but it also influences the rest of the microbiota (Correa-Fiz et al., 2019). When perinatal antimicrobials are removed from piglets, higher bacterial diversity in the nasal

Table 2
Antimicrobial resistance in *G. parasuis* reported in high percentage of isolates. Clinical and Laboratory Standards Institute (CSLI) breakpoints were used to classify isolates as resistant.

High antimicrobial resistance for *G. parasuis*			Country (number of isolates)	Reference
High (20.0–50.0%)	Very High (50.0–70.0%)	Extremely High (> 70.0%)		
–	–	–	Denmark (n = 52)	Aestroup et al., 2004
–	–	–	Germany (n = 123[a])	Brodgen et al., 2018
Gentamycin (26.7 %), Neomycin (33.3%), Spectinomycin (23.3%) Oxytetracycline (40%), Tilmicosin (40%), Enrofloxacin (20%)	Penicillin (60%), Ampicillin (56.7%) Trimethoprim/ sulphamethoxazole (53.3%)	–	Spain (n = 30)	De la Fuente et al., 2007
Neomycin (20%)	–	–	United Kingdom (n = 30)	De la Fuente et al., 2007
–	–	–	Europe (n = 68)	El Garch et al., 2015
–	–	–	United States (n = 1615)	Hayer et al., 2020
Lincomycin (42%), Tiamulin (24%), Tyalosin tartrate (20%)	–	Bacitracin (88%),Gentamycin (82%)	Brazil (n = 50)	Miani et al., 2017
Tetracycline (27.7%)	–	–	Czech Republic (n = 83)	Nedbalcova and Kučerová., 2013
Norfloxacin (49.1%), Ciprofloxacin (49.1%), Erythromycin (44.36%), Gentamycin (39.1%), Penicillin (30%), Levofloxacin (24.6%) Ampicillin (23.6%), Amoxicillin (20.9%)	Enrofloxacin (70%)	Sulfanilamide (100%), Nalidixic acid (96.4%), Trimethoprim (90.9%)	China (n = 110)	Zhang et al., 2014
Amikacin (42%), Enrofloxacin (37.8%), Gentamycin (33%), Spectinomycin (30%), Kanamycin (31%), Doxycycline (26.7%)	Ofloxacin (56.6%), Ampicillin (55%), Polymyxin (54.4%), Streptomycin (54%), Ciprofloxacin (53.3%)	Clindamycin (98.9%), Spiramycin (98%), Trimethoprim (76.7%), Lincomycin (74.4%), Neomycin (74.4%)	China (n = 90)	Zhang[b] et al., 2019a
Tilmicosin (20%)	–	–	China (n = 244)	Zhang et al., 2019b
Lomefloxacin (28.67%), Ciprofloxacin (23.78%), Norfloxacin (22.38%), Levofloxacin (20.28%)	Enrofloxacin (55.94%)	Nalidixic acid (82.52%)	China (n = 143)	Zhao et al., 2018
Trimethoprim/ sulphamethoxazole (44.5%)	–	Enrofloxacin (70.9%)	China (n = 110)	Zhou et al., 2010

Aarestrup et al., 2004. Vet. Microbiol. 101, 143–146; Brogden et al., 2018. Vet. Microbiol. 217, 32–35; de la Fuente et al., 2007. Vet. Microbiol. 120, 184–191; El Garch et al., 2016. Vet. Microbiol. 194, 11–22; Hayer et al., 2020. Res. Vet. Sci. 128, 135–144; Miani et al., 2017. Pesq. Vet. Bras. 37, 1187–1192; Nedbalcová and Kučerová, 2013. Acta Vet. Brno 82, 3–7; Zhang et al., 2019a. Sci. Rep. 9, 9908; Zhang et al., 2014. Res. Vet. Sci. 97, 171–175; Zhang et al., 2019b. Vet. Microbiol. 231, 40–44; Zhao et al., 2018. PeerJ 6, e4613; Zhou et al., 2010. Vet. Microbiol. 141.168–173.

[a] 5 isolates from Netherlands and Belgium.

[b] Data from 2017.

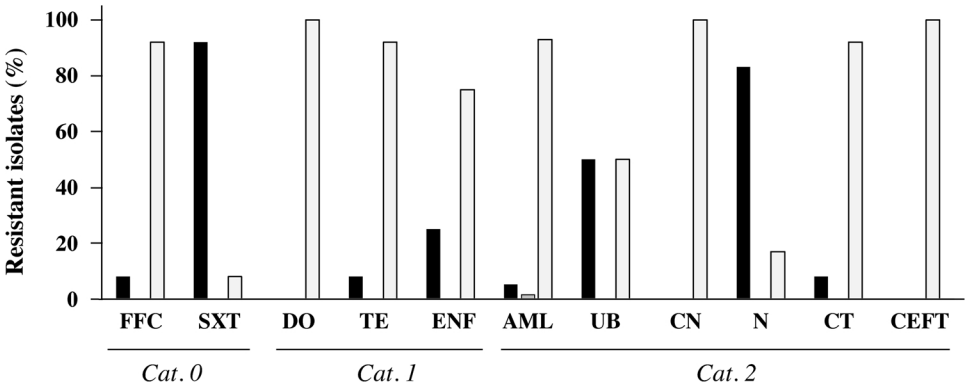

Fig. 1. Antimicrobial resistance of *Glaesserella* (*Haemophilus) parasuis* field strains isolated in HIPRA DIAGNOS laboratory between the years 2014 and 2017. Results are expressed as percentage of resistant (black bars), sensitive (white bars) and intermediate (gray bars) isolates (N = 57) to the following antimicrobials: florfenicol 30 μg (FFC), sufametoxazole-trimetroprim 25 μg (SXT), doxycycline 30 μg (DO), tetracycline 30 μg (TE), enrofloxacin 5 μg (ENF), amoxicillin 25 μg (AML), flumequine 30 μg (UB), gentamycin 10 μg (CN), neomycin 10 μg (N), colistin 10 μg (CT), ceftiofur 30 μg (CEFT). Antimicrobial categories based on Spanish Action Plan on Antimicrobial Resistance: Cat. 0, antimicrobials not included in any category since alternatives are available to treat serious human diseases and their use is not consider risky for the emergence and dissemination of resistances; Cat. 1, antibiotics used in veterinary medicine on a regular basis and as 1st choice but they have recommendations of use since they are critically important for human health; Cat. 2, antibiotics that should be used in veterinary medicine as 2nd choice and/or last resort for being antimicrobials critically important for human health (Cat. 3 corresponds to antimicrobials not permitted in veterinary medicine).

microbiota is observed, which has been linked to a better health status and well-being (Correa-Fiz et al., 2019). Antimicrobials have detrimental effects on healthy microbiota communities, and its early use in piglets induces a reduced bacterial diversity that causes a poorer performance of the immune system (Schokker et al., 2014; Correa-Fiz et al., 2019). Reduction in the use of antimicrobials, especially when treating piglets in a metaphylactic manner, should be a priority in pig production. Improvement in biosecurity and feed quality, as well as increase in the use of vaccines have been identified as promising alternatives to antimicrobials in industrial pig farming (Postma et al., 2015; Collineau et al., 2017).

To sum up, strategic antimicrobial treatments may only be advised on a few limited situations, mainly to treat piglets during a disease outbreak, which is important not only for health but also for welfare issues. Alternative control measures should be taken to minimize the potential increase of Glässer's disease caused by resistant *G. parasuis*.

4.3. Vaccination

4.3.1. Role of antibodies in protection

G. parasuis is part of the nasal microbiota. Newborn piglets are colonized upon contact with the sows during lactation, while receiving maternally derived antibodies through the colostrum, establishing a balance between colonization and immunity. *G. parasuis* is an extracellular pathogen, hence the humoral immune response (antibodies) plays an essential role in protection against disease (Nedbalcova et al., 2011). Antibodies against *G. parasuis* opsonize bacteria for subsequent detection and uptake by alveolar macrophages for killing (Olvera et al., 2009). Opsonization by antibodies seems to have less impact in complement killing (Cerdà-Cuéllar and Aragon, 2008), but the role of specific antibodies in serum susceptibility cannot be ruled out, as observed with sera obtained from pigs experimentally vaccinated or infected (Brockmeier et al., 2013). Strikingly, piglets with low antibody levels (considered negative as measured by commercial ELISA-INgezim *Haemophilus*) and with no detectable nasal colonization by *G. parasuis*, as detected by PCR, can be resistant to infection by a highly virulent strain (V. Aragon, unpublished observations). This may be explained by the presence of antibodies that are not detected in the ELISA used or by the existence of other mechanisms of protection.

4.3.2. Types of vaccines and their limitations

Most of the *G. parasuis* vaccines that are commercially available nowadays are inactivated and serovar-specific, with limited cross-protection against all pathogenic serovars (reviewed in Liu et al., 2016). It is generally acknowledged that they are efficacious against homologous serovars through the production of high levels of opsonizing antibodies. However, homologous protection against the most frequent serovars, 4 and 5, has been reported to rank from full to none (Takahashi et al., 2001; Bak and Riising, 2002), indicating that, even within the same serovar, strains may differ in antigenicity. Therefore, strains to be used in commercial vaccines should be selected carefully. Literature on cross-protection is divergent, but cross-protection against serovars 4 and 5 with heterologous vaccines has been reported in some occasions (Liu et al., 2016; Zhao et al., 2017). Some commercial vaccines include more than one serovar in the formulation to enhance cross-protection. Moreover, the adjuvant of choice has been found to be important to increase efficacy, especially when the product contains multiple antigens (Xue et al., 2015). Still, when using inactivated vaccines, pigs require multiple immunizations to generate long-term protection (Liu et al., 2016). In some occasions, autogenous vaccines containing the *G. parasuis* strains present in the farm are used. These vaccines present some drawbacks, such as variable and usually lower concentration of antigen, uncertain safety since verification is not required by regulations, and difficulties in ascertain that the correct strains are included in the composition (Kirkwood et al., 2001; Liu et al., 2016). Moreover, some of the modern adjuvants are proprietary, and therefore are not available for the preparation of this type of autogenous vaccines.

Subunit vaccines contain specific antigenic molecules of *G. parasuis,* which may lead the immune response towards common epitopes present in pathogenic strains. This type of vaccines would not affect the beneficial non-virulent strains from the microbiota, which in fact might have protective effects (Brockmeier et al., 2013). Up to now, there is no commercial subunit vaccines to prevent Glässer's disease, but multiple experimental subunit vaccines have shown protection in the pig model (Olvera et al., 2011; Fu et al., 2013; Frandoloso et al., 2015; McCaig et al., 2016; Guizzo et al., 2018). Studies to examine the heterologous protection of the vaccine candidates are still needed. The type of adjuvant is also expected to be essential in vaccine design as it can modulate differently the immune response to the same antigen (Barasuol et al., 2017).

The design of subunit vaccines frequently relays on the identification of putative surface exposed or secreted molecules, by immunoproteomics in combination with genome sequencing (Martinez-Martinez et al., 2013; Li et al., 2017a). Subsequent successful recombinant expression, good protein yield for mass production, and adequate protein folding are some of the challenges to be faced. Recent recombinant proteins identified as highly immunoreactive: proteins HxuC, HxuB, TolC, LppC, and HAPS_0926 (Wen et al., 2016; Li et al., 2017c); Omp16 (Zheng et al., 2017); superoxide dismutase (Guo et al., 2017); and HbpA, OppA, HPS-04307, AfuA, and HktE (Li et al., 2017a), have shown protection in mice against lethal challenge with *G. parasuis.* These results need to be confirmed in the pig model, since some

recombinant proteins showing protection in the mouse model failed to protect pigs (Alvarez-Estrada et al., 2018).

The identification of specific epitopes is key to design a targeted immune response against virulent *G. parasuis*. The surface expose F4 fragment, common to VtaAs of virulent strains (Correa-Fiz et al., 2017), portions of the loops of the neuraminidase (Bregon-Villahoz et al., 2017) or fragments of transferring binding proteins (TbpAB) (Frandoloso et al., 2011) are some examples of promising candidates. However, further research is needed to identify common epitopes and to prove heterologous protection in the pig model.

Full understanding of the virulence factors associated with *G. parasuis* infection will be key to improving vaccines; *e.g.* vaccines to enhance mucosal immunity and block the early steps of infection in the upper respiratory tract, would further limit the spread to internal organs. Additional considerations for vaccine design, such as the short window available for piglet vaccination, impulse the development of one-shot vaccines, possibly through research on new safe effective adjuvants for neonates or different routes of vaccination.

4.3.3. Maternal immunity and sow vaccination

The prevalence of Glässer's disease is higher in nursery piglets, and therefore management, including vaccination, should be directed to wean protected piglets (Aragon et al., 2019). Piglets are colonized by *G. parasuis* while getting protective colostrum from their dams. Maternally derived antibodies (MDA) decrease during lactation to low titters at weaning, which is commonly performed at 3–4 weeks of age (Cerdà-Cuéllar et al., 2010). The window for piglet vaccination is short, and therefore sow vaccination may be considered. There is not a complete agreement on the interference of the MDA with piglet vaccination against *G. parasuis*. While some studies report no effective interference when vaccinating piglets with two doses (Oh et al., 2013), other studies report an interference effect after sow vaccination that precluded piglet vaccination effect (Pomorska-Mol et al., 2011). This might indicate that interference could be associated to the level of MDA in the colostrum. It is worth mentioning that the use of different vaccines and serological tests in previously published studies, could also account for part of the differences in the level of interference observed. A particular case is the vaccination of naïve gilts, since this would provide protection not only to the piglets but also to the gilts themselves when facing farms with high *G. parasuis* pressure. Thus, vaccination of gilts against *G. parasuis* can be considered part of the gilt acclimation program.

Colonization at weaning is a key element that could be beneficial to avoid subsequent Glässer's disease in the farms (Correa-Fiz et al., 2016). However, there is no consensus on the influence of MDA on nasal colonization. Brean et al. (2016) found that litters of primiparous sows were colonized earlier by *G. parasuis* than those of multiparous sows, in agreement with the lower maternal immunity expected in gilts. However, other authors have reported higher isolation of *G. parasuis* from the nasal cavities of 2 week old piglets from multiparous sows, while none of the piglets from primiparous were positive (Kirkwood et al., 2001). When sow vaccination is considered, contradictory results have been reported. While Kirkwood et al. (2001) found no effect of sow vaccination on *G. parasuis* colonization of piglets, Cerdà-Cuéllar et al. (2010) found that sow vaccination delayed the colonization of piglets, reduced the bacterial load and the heterogeneity of G. parasuis strains. Nonetheless, sow vaccination may be effective to protect piglets during lactation, but, as mentioned before, lasting protection may require vaccination of piglets after the farrowing phase, together with actions to ensure an early colonization.

Altogether, to make the best decisions at the farm level, it may be useful to examine the serological profile of the herd to design of a suitable vaccination program to reduce MDA interference with piglet vaccination. In this way, higher protection rates could be achieved at the time of greatest risk of disease development (Pomorska-Mol et al., 2011).

4.4. Nasal microbiota

Recent studies on the nasal microbiota of piglets have revealed the role of this microbiota in predisposition to Glässer's disease (Correa-Fiz et al., 2016). Several genera, for example from the *Bacteroidetes* and *Firmicutes* phyla, were associated with health, supporting the possibility of mining the microbiota for health promotion. In addition, elimination of perinatal antimicrobials can increase the nasal microbiota diversity, which was also linked to health (Correa-Fiz et al., 2016, 2019). Moreover, the microbiota not only can promote resistance to pathogens but can also provide an stimulation of the immune system for an enhanced vaccine response (Lynn and Pulendran, 2018), pointing towards possible dual strategies as co-adjuvant in new vaccine formulations. These results open a new direction for disease control in swine by modulation of the nasal microbiota, promoting the elimination of metaphylactic antimicrobial treatments.

5. Final remarks

In this global context of antibiotic restriction, good management, biosecurity strategies and tailored vaccination programs targeting virulent strains of *G. parasuis* appear to be key elements to consider in Glässer's disease control. Maternal antibody levels and nasal colonization by *G. parasuis* non-virulent strains and other beneficial bacteria need to be also taken into account. Effective control of disease will need of a holistic approach, integrating the above-mentioned elements, along with effective diagnosis of the pathogenic potential and antigenic properties of the *G. parasuis* strains. Finally, if antimicrobials are needed, susceptibility tests to antimicrobials should be performed before the use of these drugs, whose use should be limited and with the narrowest spectrum of action possible.

Synergies between industry and research institutions will open the door to new opportunities, such as vaccine design with broader heterologous protection, which would lead to the future of a cost-effective, responsable and sustainable farming.

Funding

This work was partially supported by HIPRA. MCH is a recipient of a Juan de la Cierva fellowship from MINECO (Spain). The Centres de Recerca de Catalunya (CERCA) Programme is acknowledged.

Declaration of Competing Interest

EBV and JM are employees of HIPRA, a company that commercializes a vaccine against Glässer's disease. HIPRA has provided data on vaccine sales and on antibiotic resistances from its diagnostics laboratory. HIPRA has not influenced the writing of this review.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.vetmic.2020.108595.

References

Alvarez-Estrada, A., Martinez-Martinez, S., Martin, C.G., Garcia-Iglesias, M.J., Perez-Martinez, C., Yubero-Delgado, S., Guizzo, J.A., Frandoloso, R., Rodriguez-Ferri, E.F., 2018. Immunogenic characterization of vaccines based on *Haemophilus parasuis* Nagasaki strain, OmpP2, OmpP5 and OmpD15, in colostrum-deprived pigs experimentally challenged with the same strain. Res. Vet. Sci. 119, 292–301.

Angen, Ø., Oliveira, S., Ahrens, P., Svensmark, B., Leser, T.D., 2007. Development of an improved species specific PCR test for detection of *Haemophilus parasuis*. Vet. Microbiol. 119, 266–276.

Aragon, V., Cerda-Cuellar, M., Fraile, L., Mombarg, M., Nofrarias, M., Olvera, A., Sibila, M., Solanes, D., Segales, J., 2010. Correlation between clinico-pathological outcome and typing of *Haemophilus parasuis* field strains. Vet. Microbiol. 142, 387–393.

Aragon, V., Segales, J., Tucker, A.W., 2019. Glässer's disease. In: Zimmerman, J.J.,

Karriker, L.A., Ramirez, A., Schwartz, K.J., Stevenson, G.W., Zhang, J. (Eds.), Diseases of Swine. Wiley-Blackwell, New Jersey, pp. 844–853.
Bak, H., Riising, H.J., 2002. Protection of vaccinated pigs against experimental infections with homologous and heterologous *Haemophilus parasuis*. Vet. Rec. 151, 502–505.
Barasuol, B.M., Guizzo, J.A., Fegan, J.E., Martinez-Martinez, S., Rodriguez-Ferri, E.F., Gutierrez-Martin, C.B., Kreutz, L.C., Schryvers, A.B., Frandoloso, R., 2017. New insights about functional and cross-reactive properties of antibodies generated against recombinant TbpBs of *Haemophilus parasuis*. Sci. Rep. 7, 10377.
Bello-Orti, B., Costa-Hurtado, M., Martinez-Moliner, V., Segales, J., Aragon, V., 2014. Time course *Haemophilus parasuis* infection reveals pathological differences between virulent and non-virulent strains in the respiratory tract. Vet. Microbiol. 170, 430–437.
Bouchet, B., Vanier, G., Jacques, M., Auger, E., Gottschalk, M., 2009. Studies on the interactions of *Haemophilus parasuis* with porcine epithelial tracheal cells: limited role of LOS in apoptosis and pro-inflammatory cytokine release. Microb. Pathog. 46, 108–113.
Brean, M., Abraham, S., Hebart, M., Kirkwood, R.N., 2016. Influence of parity of birth and suckled sows on piglet nasal mucosal colonization with *Haemophilus parasuis*. Can. Vet. J. 57, 1281–1283.
Bregon-Villahoz, M., Gutierrez-Martin, C.B., Alvarez-Estrada, A., Rodriguez-Ferri, E.F., Frandoloso, R., Martinez-Martinez, S., 2017. Molecular study of an outer fragment of *Haemophilus parasuis* neuraminidase and utility with diagnostic and immunogen purposes. Res. Vet. Sci. 115, 463–469.
Brockmeier, S.L., Loving, C.L., Mullins, M.A., Register, K.B., Nicholson, T.L., Wiseman, B.S., Baker, R.B., Kehrli Jr., M.E., 2013. Virulence, transmission, and heterologous protection of four isolates of *Haemophilus parasuis*. Clin. Vaccine Immunol. 20, 1466–1472.
Brogden, S., Pavlović, A., Tegeler, R., Kaspar, H., De Vaan, N., Kehrenberg, C., 2018. Antimicrobial susceptibility of *Haemophilus parasuis* isolates from Germany by use of a proposed standard method for harmonized testing. Vet. Microbiol. 217, 32–35.
Castilla, K.S., de Gobbi, D.D., Moreno, L.Z., Paixao, R., Coutinho, T.A., dos Santos, J.L., Moreno, A.M., 2012. Characterization of *Haemophilus parasuis* isolated from Brazilian swine through serotyping, AFLP and PFGE. Res. Vet. Sci. 92, 366–371.
Cerdà-Cuéllar, M., Aragon, V., 2008. Serum-resistance in *Haemophilus parasuis* is associated with systemic disease in swine. Vet. J. 175, 384–389.
Cerdà-Cuéllar, M., Naranjo, J.F., Verge, A., Nofrarias, M., Cortey, M., Olvera, A., Segales, J., Aragon, V., 2010. Sow vaccination modulates the colonization of piglets by *Haemophilus parasuis*. Vet. Microbiol. 145, 315–320.
Collineau, L., Rojo-Gimeno, C., Leger, A., Backhans, A., Loesken, S., Nielsen, E.O., Postma, M., Emanuelson, U., Beilage, E.G., Sjolund, M., Wauters, E., Stark, K.D.C., Dewulf, J., Belloc, C., Krebs, S., 2017. Herd-specific interventions to reduce antimicrobial usage in pig production without jeopardising technical and economic performance. Prev. Vet. Med. 144, 167–178.
Correa-Fiz, F., Fraile, L., Aragon, V., 2016. Piglet nasal microbiota at weaning may influence the development of Glasser's disease during the rearing period. BMC Genomics 17, 404.
Correa-Fiz, F., Galofre-Mila, N., Costa-Hurtado, M., Aragon, V., 2017. Identification of a surface epitope specific of virulent strains of *Haemophilus parasuis*. Vet. Microbiol. 198, 116–120.
Correa-Fiz, F., Goncalves Dos Santos, J.M., Illas, F., Aragon, V., 2019. Antimicrobial removal on piglets promotes health and higher bacterial diversity in the nasal microbiota. Sci. Rep. 9, 6545.
Costa-Hurtado, M., Olvera, A., Martinez-Moliner, V., Galofre-Mila, N., Martinez, P., Dominguez, J., Aragon, V., 2013. Changes in macrophage phenotype after infection of pigs with *Haemophilus parasuis* strains with different levels of virulence. Infect. Immun. 81, 2327–2333.
del Rio, M.L., Gutierrez, B., Gutierrez, C.B., Monter, J.L., Rodriguez Ferri, E.F., 2003. Evaluation of survival of *Actinobacillus pleuropneumoniae* and *Haemophilus parasuis* in four liquid media and two swab specimen transport systems. Am. J. Vet. Res. 64, 1176–1180.
Dickerman, A., Bandara, A.B., Inzana, T.J., 2019. Phylogenomic analysis of Haemophilus parasuis and proposed reclassification to *Glaesserella parasuis*, gen. nov., comb. nov. Int. J. Syst. Evol. Microbiol. https://doi.org/10.1099/ijsem.0.003730.
Frandoloso, R., Martinez, S., Rodriguez-Ferri, E.F., Garcia-Iglesias, M.J., Perez-Martinez, C., Martinez-Fernandez, B., Gutierrez-Martin, C.B., 2011. Development and characterization of protective *Haemophilus parasuis* subunit vaccines based on native proteins with affinity to porcine transferrin and comparison with other subunit and commercial vaccines. Clin. Vaccine Immunol. 18, 50–58.
Frandoloso, R., Martinez-Martinez, S., Gutierrez-Martin, C.B., Rodriguez-Ferri, E.F., 2012. *Haemophilus parasuis* serovar 5 Nagasaki strain adheres and invades PK-15 cells. Vet. Microbiol. 154, 347–352.
Frandoloso, R., Martinez-Martinez, S., Calmettes, C., Fegan, J., Costa, E., Curran, D., Yu, R.H., Gutierrez-Martin, C.B., Rodriguez-Ferri, E.F., Moraes, T.F., Schryvers, A.B., 2015. Nonbinding site-directed mutants of transferrin binding protein B exhibit enhanced immunogenicity and protective capabilities. Infect. Immun. 83, 1030–1038.
Fu, S., Zhang, M., Xu, J., Ou, J., Wang, Y., Liu, H., Liu, J., Chen, H., Bei, W., 2013. Immunogenicity and protective efficacy of recombinant *Haemophilus parasuis* SH0165 putative outer membrane proteins. Vaccine 31, 347–353.
Galofre-Mila, N., Correa-Fiz, F., Lacouture, S., Gottschalk, M., Strutzberg-Minder, K., Bensaid, A., Pina-Pedrero, S., Aragon, V., 2017. A robust PCR for the differentiation of potential virulent strains of *Haemophilus parasuis*. BMC Vet. Res. 13, 124.
Guizzo, J.A., Chaudhuri, S., Prigol, S.R., Yu, R.H., Dazzi, C.C., Balbinott, N., Frandoloso, G.P., Kreutz, L.C., Frandoloso, R., Schryvers, A.B., 2018. The amino acid selected for generating mutant TbpB antigens defective in binding transferrin can compromise the *in vivo* protective capacity. Sci. Rep. 8, 7372.
Guo, L., Xu, L., Wu, T., Fu, S., Qiu, Y., Hu, C.A., Ren, X., Liu, R., Ye, M., 2017. Evaluation of recombinant protein superoxide dismutase of *Haemophilus parasuis* strain SH0165 as vaccine candidate in a mouse model. Can. J. Microbiol. 63, 312–320.
Howell, K.J., Peters, S.E., Wang, J., Hernandez-Garcia, J., Weinert, L.A., Luan, S.L., Chaudhuri, R.R., Angen, O., Aragon, V., Williamson, S.M., Parkhill, J., Langford, P.R., Rycroft, A.N., Wren, B.W., Maskell, D.J., Tucker, A.W., Consortium, B.R.T., 2015. Development of a multiplex PCR assay for rapid molecular serotyping of *Haemophilus parasuis*. J. Clin. Microbiol. 53, 3812–3821.
Howell, K.J., Weinert, L.A., Peters, S.E., Wang, J., Hernandez-Garcia, J., Chaudhuri, R.R., Luan, S.L., Angen, O., Aragon, V., Williamson, S.M., Langford, P.R., Rycroft, A.N., Wren, B.W., Maskell, D.J., Tucker, A.W., 2017. "Pathotyping" Multiplex PCR Assay for *Haemophilus parasuis*: a tool for prediction of virulence. J. Clin. Microbiol. 55, 2617–2628.
Hua, K., Li, Y., Zhou, H., Hu, X., Chen, Y., He, R., Luo, R., Zhou, R., Bi, D., Jin, H., 2018. *Haemophilus parasuis* infection disrupts adherens junctions and initializes EMT dependent on canonical Wnt/beta-catenin signaling pathway. Front. Cell. Infect. Microbiol. 8, 324.
Kielstein, P., Rapp-Gabrielson, V.J., 1992. Designation of 15 serovars of *Haemophilus parasuis* on the basis of immunodiffusion using heat-stable antigen extracts. J. Clin. Microbiol. 30, 862–865.
Kirkwood, R.N., Rawluk, S.A., Cegielski, A.C., Otto, A.J., 2001. Effect of pig age and autogenous sow vaccination on nasal mucosal colonization of pigs by *Haemophilus parasuis*. J. Swine Health Prod. 9, 77–79.
Li, B., Zhang, Y., Wei, J., Shao, D., Liu, K., Shi, Y., Qiu, Y., Ma, Z., 2015. Characterization of a novel small plasmid carrying the florfenicol resistance gene floR in *Haemophilus parasuis*. J. Antimicrob. Chemother. 70, 3159–3161.
Li, G., Xie, F., Li, J., Liu, J., Li, D., Zhang, Y., Langford, P.R., Li, Y., Liu, S., Wang, C., 2017a. Identification of novel *Haemophilus parasuis* serovar 5 vaccine candidates using an immunoproteomic approach. J. Proteomics 163, 111–117.
Li, J., Wang, S., Li, C., Wang, C., Liu, Y., Wang, G., He, X., Hu, L., Liu, Y., Cui, M., Bi, C., Shao, Z., Wang, X., Xiong, T., Cai, X., Huang, L., Weng, C., 2017b. Secondary *Haemophilus parasuis* infection enhances highly pathogenic porcine reproductive and respiratory syndrome virus (HP-PRRSV) infection-mediated inflammatory responses. Vet. Microbiol. 204, 35–42.
Li, M., Cai, R.J., Song, S., Jiang, Z.Y., Li, Y., Gou, H.C., Chu, P.P., Li, C.L., Qiu, H.J., 2017c. Evaluation of immunogenicity and protective efficacy of recombinant outer membrane proteins of *Haemophilus parasuis* serovar 5 in a murine model. PLoS One 12, e0176537.
Liu, H., Xue, Q., Zeng, Q., Zhao, Z., 2016. *Haemophilus parasuis* vaccines. Vet. Immunol. Immunopathol. 180, 53–58.
Lynn, D.J., Pulendran, B., 2018. The potential of the microbiota to influence vaccine responses. J. Leukoc. Biol. 103, 225–231.
Ma, L., Wang, L., Chu, Y., Li, X., Cui, Y., Chen, S., Zhou, J., Li, C., Lu, Z., Liu, J., Liu, Y., 2016. Characterization of Chinese *Haemophilus parasuis* isolates by traditional serotyping and molecular serotyping methods. PLoS One 11, e0168903.
Macedo, N., Cheeran, M.C., Rovira, A., Holtcamp, A., Torremorell, M., 2017. Effect of enrofloxacin on *Haemophilus parasuis* infection, disease and immune response. Vet. Microbiol. 199, 91–99.
Martinez-Martinez, S., Frandoloso, R., Rodriguez Ferri, E.F., Gil, C., Hernandez-Haro, C., Yubero, S., Gutierrez Martin, C.B., 2013. Immunoproteomic analysis of the protective response obtained with subunit and commercial vaccines against Glasser's disease in pigs. Vet. Immunol. Immunopathol. 151, 235–247.
McCaig, W.D., Loving, C.L., Hughes, H.R., Brockmeier, S.L., 2016. Characterization and vaccine potential of outer membrane vesicles produced by *Haemophilus parasuis*. PLoS One 11, e0149132.
Moleres, J., Santos-Lopez, A., Lazaro, I., Labairu, J., Prat, C., Ardanuy, C., Gonzalez-Zorn, B., Aragon, V., Garmendia, J., 2015. Novel blaROB-1-bearing plasmid conferring resistance to beta-lactams in *Haemophilus parasuis* isolates from healthy weaning pigs. Appl. Environ. Microbiol. 81, 3255–3267.
Mullins, M.A., Register, K.B., Bayles, D.O., Butler, J.E., 2011. *Haemophilus parasuis* exhibits IgA protease activity but lacks homologs of the IgA protease genes of *Haemophilus influenzae*. Vet. Microbiol. 153, 407–412.
Nedbalcova, K., Kucerova, Z., Krejci, J., Tesarik, R., Gopfert, E., Kummer, V., Leva, L., Kudlackova, H., Ondriasova, R., Faldyna, M., 2011. Passive immunisation of post-weaned piglets using hyperimmune serum against experimental *Haemophilus parasuis* infection. Res. Vet. Sci. 91, 225–229.
Oh, Y., Han, K., Seo, H.W., Park, C., Chae, C., 2013. Program of vaccination and antibiotic treatment to control polyserositis caused by *Haemophilus parasuis* under field conditions. Can. J. Vet. Res. 77, 183–190.
Olvera, A., Ballester, M., Nofrarias, M., Sibila, M., Aragon, V., 2009. Differences in phagocytosis susceptibility in *Haemophilus parasuis* strains. Vet. Res. 40, 24.
Olvera, A., Pina, S., Perez-Simo, M., Aragon, V., Segales, J., Bensaid, A., 2011. Immunogenicity and protection against *Haemophilus parasuis* infection after vaccination with recombinant virulence associated trimeric autotransporters (VtaA). Vaccine 29, 2797–2802.
Palzer, A., Ritzmann, M., Wolf, G., Heinritzi, K., 2008. Associations between pathogens in healthy pigs and pigs with pneumonia. Vet. Rec. 162, 267–271.
Pereira, D.A., Dalla Costa, F.A., Ferroni, L.B., Moraes, C.N., Schocken-Iturrino, R.P., Oliveira, L.G., 2017. The challenges with Glässer's disease in technified pig production. Aust. J. Vet. Sci. 49, 63–69.
Pomorska-Mol, M., Markowska-Daniel, I., Rachubik, J., Pejsak, Z., 2011. Effect of maternal antibodies and pig age on the antibody response after vaccination against Glässer's disease. Vet. Res. Commun. 35, 337–343.
Pomorska-Mol, M., Dors, A., Kwit, K., Czyzewska-Dors, E., Pejsak, Z., 2017. Coinfection modulates inflammatory responses, clinical outcome and pathogen load of H1N1 swine influenza virus and *Haemophilus parasuis* infections in pigs. BMC Vet. Res. 13, 376.

Postma, M., Stark, K.D., Sjolund, M., Backhans, A., Beilage, E.G., Losken, S., Belloc, C., Collineau, L., Iten, D., Visschers, V., Nielsen, E.O., Dewulf, J., Consortium, M., 2015. Alternatives to the use of antimicrobial agents in pig production: a multi-country expert-ranking of perceived effectiveness, feasibility and return on investment. Prev. Vet. Med. 118, 457–466.

Prüller, S., Turni, C., Blackall, P.J., Beyerbach, M., Klein, G., Kreienbrock, L., Strutzberg-Minder, K., Kaspar, H., Meemken, D., Kehrenberg, C., 2016. Towards a standardized method for broth microdilution susceptibility testing of *Haemophilus parasuis*. J. Clin. Microbiol. 55, 264–273.

Schokker, D., Zhang, J., Zhang, L.L., Vastenhouw, S.A., Heilig, H.G., Smidt, H., Rebel, J.M., Smits, M.A., 2014. Early-life environmental variation affects intestinal microbiota and immune development in new-born piglets. PLoS One 9, e100040.

Takahashi, K., Naga, S., Yagihashi, T., Ikehata, T., Nakano, Y., Senna, K., Maruyama, T., Murofushi, J., 2001. A cross-protection experiment in pigs vaccinated with *Haemophilus parasuis* serovars 2 and 5 bacterins, and evaluation of a bivalent vaccine under laboratory and field conditions. J. Vet. Med. Sci. 63, 487–491.

Turni, C., Singh, R., Blackall, P.J., 2018. Virulence-associated gene profiling, DNA fingerprinting and multilocus sequence typing of *Haemophilus parasuis* isolates in Australia. Aust. Vet. J. 96, 196–202.

Wen, Y., Yan, X., Wen, Y., Cao, S., He, L., Ding, L., Zhang, L., Zhou, P., Huang, X., Wu, R., Wen, X., 2016. Immunogenicity of the recombinant HxuCBA proteins encoded by hxuCBA gene cluster of *Haemophilus parasuis* in mice. Gene 591, 478–483.

Xue, Q., Zhao, Z., Liu, H., Chen, K., Xue, Y., Wang, L., 2015. First comparison of adjuvant for trivalent inactivated *Haemophilus parasuis* serovars 4, 5 and 12 vaccines against Glasser's disease. Vet. Immunol. Immunopathol. 168, 153–158.

Zhang, J., Xu, C., Guo, L., Shen, H., Deng, X., Ke, C., Ke, B., Zhang, B., Li, A., Ren, T., Liao, M., 2012. Prevalence and characterization of genotypic diversity of *Haemophilus parasuis* isolates from southern China. Can. J. Vet. Res. 76, 224–229.

Zhang, J.S., Xia, Y.T., Zheng, R.C., Liang, Z.Y., Shen, Y.J., Li, Y.F., Nie, M., Gu, C., Wang, H., 2018. Characterisation of a novel plasmid containing a florfenicol resistance gene in *Haemophilus parasuis*. Vet. J. 234, 24–26.

Zhang, P., Zhang, C., Aragon, V., Zhou, X., Zou, M., Wu, C., Shen, Z., 2019. Investigation of *Haemophilus parasuis* from healthy pigs in China. Vet. Microbiol. 231, 40–44.

Zhao, Z., Liu, H., Xue, Y., Chen, K., Liu, Z., Xue, Q., Wang, C., 2017. Analysis of efficacy obtained with a trivalent inactivated *Haemophilus parasuis* serovars 4, 5, and 12 vaccine and commercial vaccines against Glässer's disease in piglets. Can. J. Vet. Res. 81, 22–27.

Zheng, X., Yang, X., Li, X., Qiu, G.H., Dai, A., Huang, Q., Huang, C., Guo, X., 2017. Omp16-based vaccine encapsulated by alginate-chitosan microspheres provides significant protection against *Haemophilus parasuis* in mice. Vaccine 35, 1417–1423.