Guest Editorial

Understanding the virulence of *Haemophilus parasuis*

Haemophilus parasuis is the causative agent of a disease, Glässer's disease, that was once regarded as a sporadic disease of young stressed pigs (Rapp-Gabrielson et al., 2006). However, there is no doubt that modern pig production systems have resulted in the emergence of this agent as a major cause of economic loss to pig industries around the world (Aragon et al., 2012). This emergence of a once sporadic disease has highlighted the need to understand the virulence mechanisms of this pathogen. A timely review by Drs Mar Costa-Hurtado and Virginia Aragon of the Universitat Autònoma de Barcelona, published in this issue of *The Veterinary Journal*, provides a comprehensive overview of our current knowledge of the virulence factors of *H. parasuis* (Costa-Hurtado and Aragon, 2013).

While the review makes clear that there has been considerable research in the area, it is clear that progress towards a more complete understanding of the virulence mechanisms of this key pathogen has been limited by a number of issues: 1) a very difficult challenge model; 2) the diversity of isolates present as both colonisers of the upper respiratory tract and in disease outbreaks; and 3) the range in virulence seen in 'virulent' *H. parasuis* isolates. The result of these limitations, as is clearly shown in the review by Costa-Hurtado and Aragon (2013), is that there are few factors that are clearly and definitely associated with virulence.

The ability to perform challenge trials in a realistic and practical setting is a key capacity. It is interesting to note that close relatives of *H. parasuis*, such as *Avibacterium paragallinarum* and *Actinobacillus pleuropneumoniae*, are organisms in which challenge

models are easy to perform, use an upper respiratory tract challenge and involve conventional naïve animals, as shown in studies from our group (Tumamao et al., 2004; Gong et al., 2013). In contrast, the models used to reproduce Glässer's disease are difficult to use, involve unrealistic challenge methods that by-pass typical upper respiratory tract defences or use animals lacking a normal immune capacity. The models have included intra-peritoneal injection of specific-pathogen-free pigs (Kielstein and Rapp-Gabrielson, 1992), intranasal challenge of snatch farrowed colostrum deprived piglets (Aragon et al., 2010) or intra-tracheal challenge of snatch farrowed colostrum deprived piglets (Turni and Blackall, 2007).

Falkow (1988) introduced the concept of 'molecular Koch's postulates' (identifying a potential virulence-linked gene, knocking out that gene and then showing that the mutant has no or reduced virulence, while the restored mutant returns to full virulence), the application of which has provided significant insight into many pathogens (Falkow, 2004). With *H. parasuis*, studies seeking to utilise an approach such as the molecular Koch's postulates to confirm the role of a virulence factor have a major problem: the difficulty performing pathogenicity trials. There is no doubt that this challenge is major reason why we have so little definitive knowledge on the virulence factors of *H. parasuis*. While some studies have elected to use a mouse model (presumably to overcome the difficulty and expense of the available pig models), this model is not particularly relevant. Morozumi et al. (1982) showed that *H. parasuis* can cause death but with few, if any, lesions in a mouse model.

As noted in the review by Costa-Hurtado and Aragon (2013), the literature contains many studies comparing and contrasting 'virulent' and 'non-virulent' strains and isolates. Since pathogenicity screening is so difficult, these studies on potential virulence mechanisms often have to compare and contrast nasal isolates from herds/animals with no history of

clinical Glässer's disease (assumed to be non-virulent) and isolates obtained from normally sterile sites in disease pigs (assumed to be virulent); for example, the study on serum resistance by Cerdà-Cuéllar and Aragon (2008). While such comparative studies have advanced our understanding, there are clear limitations to such studies. Multiple genotypes/serovars of *H. parasuis* can be present in the upper respiratory tract of a pig (Turni and Blackall, 2010). In addition, multiple genotypes/serovars can be present in isolates obtained from diseased pigs within a farm (Oliveira et al., 2003). Clearly, under these circumstances, assigning isolates to 'virulent' and 'non-virulent' categories by site source and herd clinical history will result in misclassification of some isolates (in both categories). As an additional issue, the diverse genetic backgrounds of such collections of isolates means that the clear elegance possible in the classic molecular Koch's postulates is missing in such group comparisons.

A final issue that has made work on the virulence mechanisms of *H. parasuis* so challenging is the variation in virulence across 'virulent' isolates. A simple dichotomy, 'virulent' and 'non-virulent' does not reflect the complexity of the situation seen in the field and in experimental infections. From the work of Kielstein and Rapp-Gabrielson (1992) onwards, it has been clear that there are degrees of virulence in 'virulent' *H. parasuis*. As an example, we examined two *H. parasuis strains* (H425 and HS1387) in a catch-farrow, colostrum deprived pig model (Turni and Blackall, 2007). While both strains were virulent, there were major differences in the outcomes; the H425 strain caused a disease that progressed so rapidly that all seven pigs had to be euthanased within 4 days. In contrast, 7/9 pigs given HS1387 survived the experiment. While all seven pigs given strain H425 yielded *H. parasuis* from the brain, only 1/9 HS1387 infected pigs yielded *H. parasuis* from the brain. In contrast, the level of peritonitis in the HS1387 infected pigs was markedly more severe

than that seen in the H425 infected pigs (Turni and Blackall, 2007). While there is no doubt that both strains examined in our study were ‘virulent’, the different disease expressions (essentially acute septicaemia as compared with severe peritonitis within a single pig population) clearly flag that there are major differences in the virulence mechanisms within ‘virulent’ strains, as well as between ‘virulent’ and ‘non-virulent’ strains.

Such variation in disease expression is also seen in field outbreaks. While the majority of outbreaks of Glässer’s disease involve polyserositis (Rapp-Gabrielson et al., 2006), there are field reports, such as that of Peet et al. (1983), of an acute septicaemic disease with minimal serosal inflammation. Clearly, the interpretation of differing disease expression seen in different field outbreaks involves the complicating, additional factors of host variation and farm management. However, the results of the experimental infections in studies such as that of Kielstein and Rapp-Gabrielson (1992) and our own work (Turni and Blackall, 2007) is quite clear: considerable diversity exists within the concept of ‘virulence’ as applied to *H. parasuis*.

Given the above challenges, it is not at all surprising that, as noted by Costa-Hurtado and Aragon (2013), so little definitive knowledge exists on the virulence mechanisms of *H. parasuis*. An ability to reproduce the disease, with the use of colostrum-deprived piglets that have been either catch or snatch farrowed appearing to be the best current option, and an appreciation of the range in virulence within ‘virulent’ *H. parasuis*, will underpin further progress in this area.

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