An investigation of enzootic Glasser's disease in a specific-pathogen-free grower-finisher facility using restriction endonuclease analysis

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Abstract

Enzootic Glassers's disease was investigated to study the epidemiology of the disease strains on a farm where it presented a problem. Restriction endonuclease fingerprinting (REF) analysis technique was used, as all strains of *Haemophilus parasuis* are biochemically similar and many strains are biochemically untypable. After young weaned pigs were moved from farm A to farm B, Glasser's disease routinely occurred despite the use of antibiotics and a commercial bacterin. Isolates were taken from the nasal passages and from carcasses of clinically affected cases and subjected to REF analysis. *Haemophilus parasuis* was not isolated from any of the pigs on farm A, but it was isolated from 7/10 and 5/10 nasal swabs taken from farm B. Two *H. parasuis* strains isolated from clinical cases of Glasser's disease from farm B had an identical REF pattern, but were different from the nasal swabs and the *H. parasuis* strain contained in the bacterin. The subsequent use of a custom autogenous bacterin made from a clinical isolate of *H. parasuis* reduced the mortality rate on farm B. This investigation indicates that nasal isolates of *H. parasuis* are different than those causing clinical disease, and not all bacterin strains are cross protective for other strains.

Résumé

Étude de la maladie de Glasser par typage par enzymes de restriction sur une ferme d'élevage de porcs de croissance et de finition, absents de pathogènes spécifiques

Cette étude fait suite à une enquête sur les souches causales de la maladie enzootique de Glasser présente sur une ferme d'élevage. La méthode d'analyse utilisée a été par typage par enzymes de restriction puisque toutes les souches d'*Haemophilus parasuis* sont semblables au point de vue biochimique et plusieurs souches ne peuvent être typées. La maladie de Glasser se déclarait couramment lorsque les porcelets sevrés étaient transférés de la ferme A à la ferme B, et ce, malgré l'administration d'antibiotiques et d'une bactérine commerciale. Des échantillons, prélevés des voies nasales et des carcasses des animaux atteints, ont été soumis aux analyses. *Haemophilus parasuis* n'a pas été isolé à partir des échantillons provenant de la ferme A, mais a été identifié dans 12 des 20 écouvillons nasaux prélevés à la ferme B. Deux souches d'*H. parasuis* isolées à partir de cas cliniques de la maladie de Glasser provenant de la ferme B obtenaient des résultats d'analyse identiques, mais différaient des souches isolées à partir des voies nasales et de la souche d'*H. parasuis* contenu dans la bactérine. Une bactérine autogène fait à partir d'un isolat d'*H. parasuis* provenant d'un cas clinique a été administrée aux porcs et son utilisation a réduit le taux de mortalité sur la ferme B. Cette étude indique que les isolats d'*H. parasuis* des voies nasales diffèrent des souches causales de la maladie clinique et que les souches de bactérine ne fournissent pas toutes une protection croisée pour les autres souches.

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Introduction

Glasser's disease occurs only sporadically in conventionally raised swine, but it has emerged recently as an important cause of morbidity and mortality in specific-pathogen-free (SPF) pigs when they are exposed to the causative agent, *Haemophilus parasuis*. This bacterium is a common inhabitant of the upper respiratory tract of conventionally raised pigs, but infection usually remains subclinical (1). Specific-pathogen-free pigs raised in strict isolation from conventional pigs are free of *H. parasuis* infection. However, significant losses can be encountered when SPF pigs are moved to conventional facilities (1,2), or when these bacteria enter an *H. parasuis* naive herd (3).

It is difficult to trace the source of infection during an outbreak of Glasser's disease, since all strains of *H. parasuis* are biochemically similar. Serological typing is extremely difficult, because there are no standardized typing sera, and there are many untypable (autoagglutinating) strains (4). In recent years, restriction endonuclease fingerprinting (REF) analysis has been used to type a number of different pathogens (5), including field strains of *H. parasuis* (6). One of the advantages of REF is that all isolates can be typed and grouped on the basis of their REF pattern.

In this study, two separate SPF facilities belonging to the same individuals were investigated. All breeding stock and young pigs were housed on farm A, premises with no history of Glasser's disease since repopulation. When young, weaned pigs were moved to farm B (an SPF facility containing only pigs from farm A), Glasser's disease occurred routinely in the newly shipped pigs, approximately one week after their arrival. This happened in spite of introduced pigs being vaccinated with a commercial Glasser's bacterin at

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least two weeks prior to transfer. Clinical signs in affected pigs ranged from acute death to chronic lameness and unthriftiness, all of which resulted in significant economic loss to the producers.

The objective of this investigation was to determine the basis for the extreme susceptibility to Glasser's disease of farm A pigs after transfer to farm B. Nasal swabs from clinically healthy pigs from both farms and swabs from the serosal surfaces of pigs that had died of Glasser's disease on farm B were cultured for *H. parasuis*. All isolates were subjected to REF analysis to determine the distribution of strains between, and within, these two farms, and to determine if these strains were present in the commercial *H. parasuis* bacterin used on farm A.

Materials and methods

Farm history

In the summer of 1988, farm A, a 150 sow and weaner facility, was depopulated, cleaned with a high pressure washer, and disinfected with 4-chloro-3,5-xylenol-4 and isopropyl alcohol (Dettol, Rickitt and Colman Canada Inc., Lachine, Quebec). Farm B, a finishing unit holding about 900 pigs, was depopulated during the fall of 1988, cleaned with a high pressure washer, and disinfected with sodium hypochlorite (Javex, Colgate Palmolive Canada Inc., Toronto, Ontario) and copper sulfate. Both premises remained empty for at least one month prior to repopulation. Farm A was populated with gilts obtained from a primary SPF herd; the offspring of these gilts were moved to farm B at approximately eight weeks of age, starting in January 1989. Requirements for human entry to farm A included a footbath, shower, and a complete change of clothing. Admittance to farm B was restricted to personnel coming from farm A, but only footbath disinfection was required prior to entry. The two farms were approximately 10 km apart, and pigs were moved by truck from farm A to farm B only. The truck was cleaned but not disinfected between loads of pigs. Farm A was 3 km from the nearest pig farm, but farm B was located approximately 150 m from a farm that contained pigs purchased from a livestock auction.

In the summer of 1989, the swine ambulatory service at the Atlantic Veterinary College (AVC) was contacted, because the producers were concerned about the performance of pigs that had been moved from farm A to farm B. Within two weeks of arrival, weaner pigs (20 kg) were lethargic and had developed swollen joints and lameness. Further questioning of the owners revealed that they had observed clinical signs ranging from acute neurological disease to chronic unthriftiness in newly arrived pigs at farm B since its repopulation. On farm B, Glasser's disease was diagnosed on the basis of the history, clinical signs, postmortem findings, and isolation of *H. parasuis* from pigs submitted to the AVC diagnostic laboratory. During the period from May 1989 to August 1991, 13 pigs that had died suddenly on farm B were submitted to the diagnostic laboratory at the AVC (records were unavailable prior to May 1989). Pigs at farm A remained healthy prior to and during this investigation.

In an attempt to control Glasser's disease on farm B, pigs on farm A received an intramuscular injection of 4 mL of benzathine and procaine penicillin (Pendure Neat, Langford Inc., Guelph, Ontario) immediately prior to their transfer to farm B. A combination of penicillin, sulfamethazine, and tetracycline (ASP-250, Cyanamid, Willowdale, Ontario) was also administered in the feed at a dose of 2.5 kg/tonne. Commencing in July 1990, six-week-old pigs on farm A were immunized with commercial *H. parasuis* bacterin before transfer to farm B. The first dose was administered two weeks prior to transfer, and a second dose was given upon arrival at farm B. In June 1991, the commercial bacterin was replaced by a bacterin prepared from a strain of *H. parasuis* that had been isolated from a case of Glasser's disease on farm B.

Collection of specimens

The protocol for the culture of nasal swabs was a modification of a method published previously (2). In the fall of 1990, nasal swabs were taken from 10 weaned pigs, approximately eight weeks of age, on farm A. On farm B, swabs were obtained from 10 other pigs after they had been resident on farm B for three weeks. These pigs were approximately 11 to 12 weeks of age. Nasal swabs were obtained from pigs on both farms on the same day and then retested in the same manner eight weeks later.

Bacteriology

Swabs were transported in tryptone yeast extract (TYE) broth and plated in triplicate onto agar (Isotonic Sensi, Merck, Darmstadt, Germany), fortified with 5% defibrinated horse blood, 5 μg/mL bacitracin (Aldrich Chemical, Milwaukee, Wisconsin, USA), 1.5 μg/mL lincomycin (Gibco Canada, Burlington, Ontario), and 0.1 μg/mL crystal violet (Sigma, St. Louis, Missouri, USA), within three hours of collection. A streak of 1% nicotinamide dinucleotide (NAD) (Fisher Scientific, Ottawa, Ontario) was applied across the plate, perpendicular to the direction of inoculation. Cultures were incubated in a humid atmosphere at 35°C in 5% CO_2. Serosal swabs taken at postmortem from naturally occurring cases of Glasser's disease were cultured and isolates of *H. parasuis* were identified in the same manner as those from the nasal swabs. Organisms that exhibited satellite growth near the NAD streak and were mannitol −, urease −, catalase +, α-fucocidase +, and τ-aminolevulinic acid + were identified as *H. parasuis*.

Restriction endonuclease analysis

The following procedure was used to extract DNA. Colonies of *H. parasuis* were subcultured, and several of these colonies were used to heavily streak a blood agar plate. After incubation for 24 h, cells were harvested in 2 mL of saline. The cells were disrupted by the addition of 30 μL of 10% sodium diodecyl sulphate (SDS) and 25 μL of 20 μg/uL proteinase K (Boehringer Mannheim Canada, Laval, Quebec). The mixture was incubated for 24 h at 37°C. The DNA was extracted, initially, using an equal volume of phenol and, subsequently, twice using an equal volume of chloroform:isoamyl alcohol (97:3). The DNA was precipitated by the addition of NaCl to a final concentration of 0.3 M and two volumes of cold 95% ethanol. After centrifugation for 10 min in a microfuge

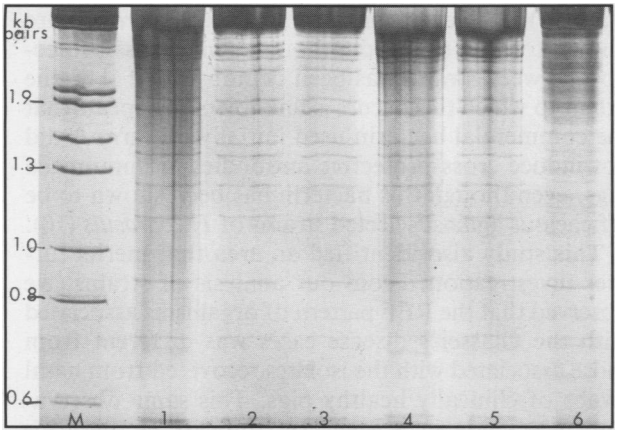

Figure 1. Polyacrylamide gel of silver stained *Bam*HI digested DNA of *Haemophilus parasuis* nasal isolates from farm B.

Lane M, molecular weight marker lambda DNA digested with *Eco*RI and *Hin*dIII, lanes 1–5, 5 isolates — pattern 1; lane 6, pattern 2.

at 13,000 × *g*, the pellets were dried and then resuspended in 200 μL 10 mM trisethylenediaminetetraacetic acid buffer. The DNA was digested using a two to tenfold excess of *Bam*HI (Boehringer Mannheim Canada) according to the manufacturer's instructions. The restriction digests included RNase A (Boehringer Mannheim Canada) at a final concentration of 10 μg/mL. Samples containing approximately 2 μg of DNA were loaded onto a 7.5% SDS polyacrylamide gel and subjected to electrophoresis using a vertical gel apparatus (Mini Protean II, Biorad, Mississauga, Ontario). The DNA bands were viewed using a modification of the silver staining method of Sammons (7).

Results

Bacteriology

Farm A — Haemophilus parasuis was not isolated from any pig on farm A. However, postmortem reports from the AVC diagnostic laboratory showed that *H. parasuis* had been isolated from the upper respiratory tract of pigs on this farm prior to repopulation.

Farm B — Haemophilus parasuis was isolated from 12 out of 20 nasal swabs from pigs on farm B. These 12 strains produced two different REF patterns, one of which predominated (shared by 11 strains). Representative strains with the predominant pattern (arbitrarily numbered as 1) are shown in lanes 1–5 of Figure 1, whereas lane 6 has a different REF pattern (arbitrarily numbered as 2). Five strains of *H. parasuis* were isolated from cases of Glasser's disease; however, only two were available for REF analysis. These two strains had identical REF patterns (data not shown), but this pattern was different from the REF patterns (1 and 2) obtained from the nasal isolates (Figure 1). The REF patterns of the nasal strains and the disease associated strains were not the same as the REF patterns of the strains of *H. parasuis* contained in the commercial bacterin (data not shown).

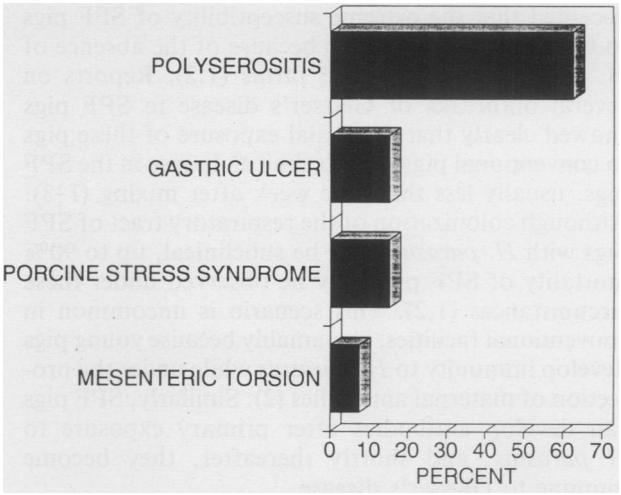

Figure 2. The proportional mortality rate on 13 necropsied pigs from farm B submitted to the diagnostic laboratory between May 1989 and August 1991.

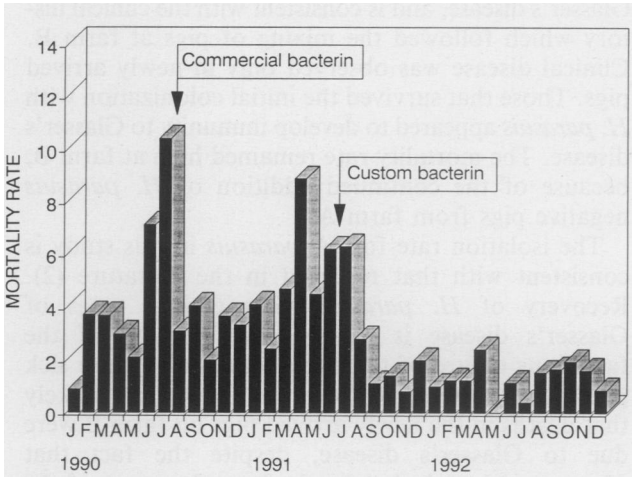

Figure 3. The monthly mortality rate expressed as percent of incoming pigs on farm B from January 1990 to December 1992, along with bacterin intervention dates.

Clinical data

The diagnosed diseases and the proportional mortality rate from pigs submitted for postmortem examination are given in Figure 2. Eight of the 13 pigs examined had gross lesions of polyserositis. Culture of the internal organs and serosal surfaces of these pigs led to the recovery of *H. parasuis* from only three pigs.

Neither vaccination with a commercial bacterin nor the administration of antibiotics, parenterally or in the feed, reduced mortality on farm B to an acceptable level. In August 1991, the mortality rate on farm B decreased markedly (Figure 3). This coincided with the arrival of pigs that had been immunized with the custom autogenous *H. parasuis* bacterin. There was no change in farm management and there were no other identified factors associated with this reduction in mortality.

Discussion

Several principles regarding the biology of *H. parasuis* and the pathogenesis of Glasser's disease in SPF pigs are shown in this investigation. It is now generally

accepted that the extreme susceptibility of SPF pigs to Glasser's disease results because of the absence of *H. parasuis* on most SPF farms (1,2). Reports on several outbreaks of Glasser's disease in SPF pigs showed clearly that the initial exposure of these pigs to conventional pigs lead to clinical disease in the SPF pigs, usually less than one week after mixing (1-3). Although colonization of the respiratory tract of SPF pigs with *H. parasuis* may be subclinical, up to 90% mortality of SPF pigs may be observed under these circumstances (1,2). This scenario is uncommon in conventional facilities, presumably because young pigs develop immunity to *H. parasuis* while under the protection of maternal antibodies (2). Similarly, SPF pigs can develop antibodies after primary exposure to *H. parasuis*, and shortly thereafter, they become immune to Glasser's disease.

In this investigation, it was shown that pigs from farm A were first exposed to *H. parasuis* upon arrival at farm B. This finding provides an explanation for the extreme susceptibility of pigs from farm A to Glasser's disease, and is consistent with the clinical history which followed the mixing of pigs at farm B. Clinical disease was observed only in newly arrived pigs. Those that survived the initial colonization with *H. parasuis* appeared to develop immunity to Glasser's disease. The mortality rate remained high at farm B, because of the continued addition of *H. parasuis* negative pigs from farm A.

The isolation rate for *H. parasuis* in this study is consistent with that reported in the literature (2). Recovery of *H. parasuis* from clinical cases of Glasser's disease is usually low, because of the fastidious nature of the bacterium and because sick pigs are often treated with antibiotics (1,2). It is likely that deaths in the newly arrived pigs on farm B were due to Glasser's disease, despite the fact that *H. parasuis* was isolated only from three out of the eight of cases of polyserositis.

The *H. parasuis* present on farm B has at least two possible sources. Close proximity of farm B to the neighboring conventional swine operation may have facilitated the transmission of *H. parasuis* between these two premises. Other studies (3,8) have shown that close proximity of SPF pigs to conventional facilities can be associated with the introduction of several swine pathogens into high health status herds. The survival of *H. parasuis* in the barn environment is another possible source of infection for pigs on farm B. Since *H. parasuis* was never recovered from farm A, it is likely that the depopulation procedures on farm A were sufficient to remove *H. parasuis* from the environment. However, on farm B, the bacterium may have survived the disinfection regime. Several factors may have contributed to the survival of *H. parasuis*. First, depopulation and washing occurred in the fall and winter when low temperatures and freezing may have preserved the organism. Second, chlorine-based disinfectants are quickly inactivated by organic matter (9), such as dried mucus or manure. Copper sulfate is a poor disinfectant (9), and its use on farm B would not have contributed greatly to elimination of bacteria.

Vaccination has been shown to be an effective method to control Glasser's disease in SPF pigs (1,10,11). However, many strains of *H. parasuis* are known to exist (6), and although some cross protection between strains has been demonstrated (11), the extent to which this occurs is unknown. It appears that the commercial bacterin used initially on farm A did not induce cross-protective antibodies in immunized pigs, even though this bacterin has been shown to be efficacious against selected strains of *H. parasuis* (10).

This study also identified an area that merits further investigation. From our analysis of strains, we observed that the REF pattern of organisms associated with the Glasser's disease cases was different from those associated with the isolates recovered from nasal swabs of clinically healthy pigs. This same observation was made in a separate study of *H. parasuis* strains associated with pigs in southern Ontario (6). It is not known if disease-causing strains are carried subclinically and, if they are, from what region of the body they could be recovered. The recovery of *H. parasuis* from nasal swabs will indicate the presence of this organism within a herd, but it does not determine if these are the strains that are involved in a disease outbreak. The technique of REF analysis will be a useful tool for clarification of this issue.

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