RESEARCHREPORT
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Title: Survey of the Molecular Epidemiology of *Haemophilus parasuis* – **NPB #02-079**

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Abstract: The objective of the present study was to characterize the genetic diversity of *Haemophilus parasuis* field isolates with regard to serovar, herd of origin, and site of isolation. Isolates of *H. parasuis* obtained from pigs in 15 North American herds and multi-farms systems were evaluated. Ninety-eight *H. parasuis* isolates were genotyped with the Enterobacterial Repetitive Intergenic Consensus (ERIC)-based polymerase chain reaction (ERIC) technique and serotyped via agar gel precipitation test. Genomic fingerprints were analyzed and dendrograms were constructed to identify strains from the same serovar group, herd of origin, or isolation site and to evaluate the genetic variability within these categories. Results showed that serovar 4 (39%) and non-typeable (NT) isolates (27%) were most prevalent. Thirty-four distinct strains were identified among the 98 isolates using a 90% similarity cutoff. Strains from serovar 4 and non-typeable isolates had high genetic diversity (12 and 18 strains, respectively). One to 3 major clusters of prevalent strains could be identified in most of the evaluated herds. *Haemophilus parasuis* strains isolated from the upper respiratory tract were either serovar 3 or NT isolates. Potentially virulent strains (isolated from systemic sites) were either serovars 1, 2, 4, 5, 12, 13, or 14, or NT isolates. Although *H. parasuis* had high genetic diversity overall, only a few strains caused disease in the studied herds. The ERIC-PCR technique was more discriminative than serotyping, and a broad genetic variety was observed within particular serovar groups.

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Introduction: *Haemophilus parasuis* is a potentially pathogenic gram-negative organism that colonizes the upper respiratory system of swine. Systemic infection caused by *H. parasuis* is characterized by fibrinous polyserositis, arthritis and meningitis. The factors involved in systemic invasion by *H. parasuis* are largely unknown. However, some studies have suggested a relationship between phenotypic and genotypic characteristics and virulence potential.[1-4.]

The peak of *H. parasuis* infections occurs in the nursery, when pigs are at 5 to 8 weeks of age.[5] Control of *H. parasuis* can be attempted by vaccination, however there is no universal vaccine for this agent so far. Considering that homologous protection is satisfactory[6] and heterologous protection between different serovars and strains is very restricted,[7] autogenous vaccination may be the best option in herds experiencing vaccination failure with commercial vaccines. The selection of a good vaccine candidate depends on the accurate characterization of the prevalent strains causing disease in affected herds. Control of infection, on the other hand, depends on the surveillance of the epidemiology of *H. parasuis* within and between related herds. The characterization of new isolates recovered from diseased pigs following vaccination allows the update of the vaccine strain(s) and detection of potential sources of pathogenic strains.

The study of the epidemiology of *H. parasuis* has been traditionally based on serotyping, [3, 8-11] which is performed using heat-stable soluble antigens and the agar gel precipitation test (AGPT).[12] Fifteen serovars of *H. parasuis* have been identified through AGPT.[3] *Haemophilus parasuis* serovars 5 and 4 have been reported as the most prevalent among isolates recovered from field cases in Japan,[2] Germany,[3] United States[8] and Spain.[10] Australian isolates have been mostly identified as serovars 5 and 13.[9,11] All studies have reported a high percentage of non-typable (NT) *H. parasuis* isolates, which ranges from 15.2%[8] to 41%.[11]

Molecular-based techniques have also been used to study the epidemiology of *H. parasuis* infections. Techniques such as restriction endonuclease fingerprinting (REF) analysis[13] and multi-locus enzyme electrophoresis (MLEE) have been successfully used to characterize and compare *H. parasuis* strains recovered from different herds and from different body sites in affected and healthy animals. The characterization of respiratory and systemic *H. parasuis* strains by MLEE, for example, revealed a high diversity among *H. parasuis* isolates, including strains from the same serovar group.[14]

Recently, another molecular-based technique, known as repetitive element based-PCR (Rep-PCR), has been successfully used to characterize the diversity of *H. parasuis* isolates. Raffie et al (2000) used the enterobacterial repetitive intergenic consensus-based PCR (ERIC-PCR) to characterize and compare *H. parasuis* isolates recovered from three different swine herds.[15] Ruiz et al. (2001) also compared the genotypic profiles of 53 isolates recovered from different body sites using ERIC-PCR, finding evidence of a clonal relationship among *H. parasuis* isolates causing systemic disease.[4]

Objectives: Considering the importance that the epidemiological features of *H. parasuis* has for disease control, the study presented here was undertaken to further characterize the diversity of *H. parasuis* field isolates by means of serotyping and genotyping. On the basis of dendrograms constructed via genomic fingerprints, genetic variability among strains within and among herds, among members of serovar groups, and among isolates obtained from respiratory tract and systemic sites was evaluated.

Materials and Methods:

Bacterial strains—Ninety-eight *H parasuis* isolates obtained from 10 swine herds and 5 multi-farm systems were genotyped and compared; these isolates were submitted to the Veterinary Diagnostic Laboratory (VDL) at the University of Minnesota between August 1999 and December 2001.

Bacterial culture and identification—*Haemophilus parasuis* isolates were cultured in chocolate agar (37°C) for 24 hours in a candle jar. Clinical specimens were initially plated on sheep blood agar with a nurse streak of *Staphylococcus aureus* and incubated (37°C) for 24 hours. Bacterial colonies with satellitism to *S aureus* were further characterized through biochemical testing. Isolates were identified as *H parasuis* on the basis of negative results of urease, aesculin, and cAMP tests, with confirmation provided by PCR assay.[16]

Serotyping—Serotyping of *H parasuis* was performed as described,[11] except that chocolate agar was used as the growth medium.

DNA extraction—Bacterial colonies were removed from growth media with an inoculation loop, suspended in 300 µL of sterile PBS solution (pH 7.0), and centrifuged at 21,000 X *g* for 5 minutes. After centrifugation, the supernatant was discarded; the remaining pellet was suspended in 200 µL of a commercially available reagent[a] and boiled for 10 minutes. After boiling, tubes were centrifuged at 13,000 X *g* for 3 minutes. Fifty microliters of the supernatant was mixed with 50 µL of sterile distilled water and stored (4°C). This final solution was used as DNA template in the PCR reaction. By means of a spectrophotometer, extracted DNA was quantified at 260 nm (1 optical density unit = 50 µg/mL).

PCR conditions—*Haemophilus parasuis* isolates were genotyped by rep-PCR assay using primers that target the ERIC sequence.[17] The assay was performed in a 25µL-reaction mixture containing 100 ng of template DNA, 1.2 µM of each primer, 2.5 µL of 10X buffer[b] (500 mM KCl and 100 mM Tris-HCl), 3 mM $MgCl_2$, 0.23 mM of each deoxynucleoside triphosphate[b], and 0.75 units of *Taq* DNA polymerase.[b] The rep-PCR assay was conducted for 30 cycles consisting of denaturation for 30 seconds (94°C), annealing for 1 minute (50°C) and extension for 2 minutes (72°C). Cycling was accomplished in a thermal cycler.[c] Twelve microliters of each reaction mixture was loaded onto an 18-cm-long 2% agarose gel; electrophoresis was performed at 70V in Tris-acetate-EDTA buffer for 3.5 hours. A 1-kb ladder was included as a size reference.[d] Gels were stained with ethidium bromide and photographed.

Computer analysis of genomic fingerprints—Gel images were stored digitally and analyzed with computer software.[e] Genomic fingerprints were analyzed by calculating the similarity matrices of whole densitometric curves of the gel tracks using the pair-wise Pearson's product-moment correlation coefficient. The rep-PCR genomic fingerprints from 200 bp to 3 kb were compared. Cluster analysis of similarity matrices was performed by the unweighted pair group method using arithmetic averages. A dendrogram containing all 98 *H parasuis* strains was constructed to assess the overall genetic diversity in this sample; assessments were also made of the genetic diversity in each serovar group, among strains within and among herds, and among isolates obtained from respiratory tract and systemic sites. The molecular epidemiologic features

and genetic diversity within herds were further evaluated by constructing separate dendrograms for herds containing ≥ 10 isolates each.

Definition of the similarity cutoff for differentiation of strains—The cutoff for strain differentiation was established by comparison of manual and computer-based assignment of strains to different groups. The 98 *H parasuis* isolates were assembled into a single dendrogram. Isolates with similar genomic fingerprints were included in the same strain group. Fingerprints were compared visually and the number of different groups of strains was defined. The similarity cutoffs of 100, 95, 90, 85, and 80% were then used to evaluate the number of strains identified by the computer software.[e] The visual identification of strains was used as the standard for the selection of the similarity cutoff to be used in the computerized differentiation of strains.

Results: Between August 1999 and December 2001, 98 *H parasuis* isolates were obtained from 10 swine herds and 5 multi-farm systems (Table 1); these were submitted to the Veterinary Diagnostic Laboratory (VDL) at the University of Minnesota for genotyping and serotyping.

Standard cutoff for strain differentiation—Thirty-eight distinct fingerprints were identified visually through comparison of bands patterns obtained with the ERIC-PCR technique. The 90% similarity cutoff identified 34 different strains, while the cutoffs of 100, 95, 85, and 80% identified 98, 46, 26, and 23 different strains, respectively. The 90% similarity cutoff was used as the standard for strain differentiation in the constructed dendrograms.

Overall genetic diversity—Genomic fingerprints obtained for 98 *H parasuis* field isolates were compared (**Fig 1**). Applying the 90% similarity cutoff, 34 strains were identified among the evaluated isolates.

Genetic diversity within serovar groups—Nine distinct serovars were detected among the evaluated *H parasuis* isolates. Serovar 4 (39%) was the most prevalent, followed by serovars 3 (8%), 1 (7%), 12 (7%), 2 (4%), 14 (3%), 5 (2%), 7 (2%), and 13 (1%). Twenty-six of 98 (27%) isolates did not react with any of the sera against the 15 known serovars of *H parasuis*, and were classified as NT. Strains from serovar 4 and NT isolates had high genetic diversity, with 12 and 18 different strains, respectively. The remaining serovar groups contained either 1 (serovar 5), 2 (serovars 1, 3, and 7), 3 (serovars 12 and 14) or 4 (serovar 2) strains. *Haemophilus parasuis* isolates from the same serovar group were clustered together in the dendrogram (**Fig 1**). In general, the genotype of an isolate was a good predictor of its serovar group, with a few exceptions. Relationships between serovar and site of isolation were noted (**Table 2**). Tonsil isolates were either serovar 3 or NT. Serovar 7 was only isolated from the lungs. Serovars 1, 4, 12, and 14, and NT isolates were obtained from lungs of pigs with pneumonia or from systemic sites. Serovars 2, 5, and 13 were only isolated from systemic sites.

Genetic diversity within and between herds—The distribution of serovars among herds was evaluated (**Table 1**). In 11 of the 15 herds, ≥ 2 isolates were obtained from affected animals. The multi-farm system C, from which 43 isolates were evaluated, had the most diverse genotype and serovar profiles, with 18 different strains and 9 serovar groups (**Table 1**, **Fig 2**). The remaining herds with > 1 isolate had either 1, 2, or 3

serovars isolated from the system, including NT isolates. Many (40%) of these herds and multi-farm systems were affected by ≥ 2 different strains.

Non-typeable strains of *H parasuis* had high genetic variability within herds, with 2 (herd N) to 5 (multi-farm system C) different strains isolated from a single herd or system. A similar degree of genetic diversity was observed for serovar 4 strains within and between herds. Although serovar-4 isolates were obtained from 40% of the herds, the distribution of different strains appeared to be herd dependent. Herd I was affected by 2 unique serovar-4 strains, which were not detected in the remaining herds (**Fig 1**). Similar results were observed for herds C and G, which had different prevalent serovar-4 strains (**Fig 2**). Serovar-2 strains were also highly diverse among herds; 4 serovar-2 strains were obtained from herds C, D, J, and M (Fig 1).

Separate dendrograms were constructed for the multi-farm systems C, D, and G to better evaluate the molecular epidemiology within systems (**Fig 2**). *Haemophilus parasuis* isolates were obtained from system C during the period of August 1999 to December 2000. This multi-farm system had several nurseries, which received pigs from multiple sources (5 to 12 sow farms). Three major clusters of genetically related *H parasuis* strains were identified in this herd. Serovar-4 strains were predominant in cluster 1, while genetically related strains that were classified either as serovars 1 or 13, or NT isolates were the main components of cluster 2. Cluster 3 included a variety of strains that were not prevalent in the system (**Fig 2**).

Multi-farm system D also had nurseries that received pigs from multiple sources. *Haemophilus parasuis* isolates obtained from this system during the period of May 2000 to December 2001 (**Fig 2**) included high diversity of strains, especially among NT strains. A cluster of closely related serovar-4 strains was prevalent in the herd throughout 16 months (August 2000 to December 2001).

Most isolates were obtained from multi-farm system G during November 2001(**Fig 2**); in the nursery at that time, there was an increase in pig deaths caused by *H parasuis* infections. The nursery that was evaluated in our study was stocked with pigs from 16 sow herds. The isolates collected were compared with isolates obtained previously from this system. Results showed that the prevalent strains that were affecting the herd during November 2001 differed from strains isolated during May and July 2001. Two major clusters were observed in the dendrogram for system G. Cluster 1 included closely related serovar-12 strains, while cluster 2 included serovar-4 strains (**Fig 2**).

Genetic diversity in isolates from various sites—*Haemophilus parasuis* isolates obtained from the upper respiratory tract differed from strains obtained from systemic sites and from lungs of pigs with pneumonia, regarding their serovars (**Table 2**) and genotype profiles (**Fig 1**). The genetic diversity among isolates from the respiratory tract and systemic sites was similar; 20 strains were isolated from lungs and 22 were obtained from systemic sites such as pleura, pericardium, brain, and joints. Strains showing similar genomic fingerprints were obtained from either lungs of pigs with pneumonia or systemic sites. Strains from lungs of pigs with pneumonia or systemic sites were not isolated from the upper respiratory tract, with the exception of isolate H 7164 8/2-Y, which was originally isolated from the nasal cavity of a healthy sow and clustered with strains obtained from lungs of pigs with pneumonia, joints, brain, and pericardium (**Fig 1**).

Discussion: In our study, the diversity of *H parasuis* field isolates was characterized serotypically and genotypically. Nine distinct serovars and 34 different genomic fingerprints were identified with AGPT and ERIC-PCR. High genetic diversity was observed within serovar groups, especially within serovars 2 (4 genotypes) and 4 (12 genotypes), and NT isolates (18 strains). Blackall et al[14] studied the diversity of 40 Australian isolates and 8 reference strains of *H parasuis* by means of MLEE. Thirty-four electrophoretic types were recognized. Considerable diversity among isolates of the same serovar was observed, indicating that serotyping is not a particular suitable technique for strain typing in epidemiological studies.[14] Our data indicated that the ERIC-PCR assay provides more accurate strain differentiation, especially within NT strains. The AGPT has traditionally been used in epidemiologic investigations of *H parasuis* infections within and among herds.[3, 6, 9-11] Information provided by this test can be used to select commercial vaccines to control *H parasuis* infections. Successful homologous protection between isolates from the same serovar group has been revealed by several studies.[6, 18-21] However, results of some studies[6, 7] suggest a lack of cross-protection among different strains from the same serovar group. Accurate strain characterization is essential for the selection of strains for inclusion in autogenous vaccines. In this instance, genotyping of field isolates by ERIC-PCR can provide more accurate strain differentiation, regardless of the serovar group.

To the authors' knowledge, this is the first study to use computer software to compare a substantial number of *H parasuis* isolates and to create a reference database for genotypic comparison of *H parasuis* strains. Results obtained in our study also provided updated information regarding the most prevalent *H parasuis* serovars isolated from US herds between 1999 and 2001. Serovar 4 (39%) was the most prevalent, followed by serovars 3 (8%), 1 (7%), 12 (4%), 2 (4%), 14 (3%), 5 (2%), 7 (2%), and 13 (1%). Non-typeable isolates were also frequently identified, accounting for 28% of the evaluated isolates. Rapp-Gabrielson and Gabrielson[8] serotyped 260 *H parasuis* isolates obtained from North American and Canadian herds during 1982 to 1990; serovars 5 (24.3%) and 4 (16.1%) were reported as the most prevalent, followed by serovars 13 (11.1%), 14 (8.6%), 2 (8.2%), 12 (6.6%), and 7 (3.7%). Serovars 1, 6, 8, 9, 11, and 15 were infrequently detected (6.2% total). Serovar 5, which has been reported to be prevalent in the US,[8] Japan,[2] Germany,[3] and Spain,[10] was only occasionally identified among the samples submitted to the VDL at the University of Minnesota during the period of 1999 to 2001. Compared with results of these other studies, the differences in the prevalence of *H parasuis* serovars observed in our study may be attributed to the different number of isolates serotyped, country of origin, and date of isolation. Our data suggested that there had been a shift in the prevalence of *H parasuis* serovars affecting US herds compared with that noted during 1982 to 1990.[3] Swine production has changed during the past years, particularly in regard to herd size, production sites, and health status of the herds. These changes could have influenced the herd distribution of *H parasuis* serovars and genotypes among US herds. However, in our study, the impact of the modernization of swine production on the epidemiologic features of *H parasuis* was not assessed.

Investigation of the relationship between serovar and site of isolation revealed that serovar 3 was mainly isolated from the upper respiratory tract, which indicates that this serovar may have low virulence. Serovars 1, 4, 12, and 14, and NT isolates were recovered either from lungs of pigs with pneumonia or from systemic sites, while serovars 2, 5, and 13 were obtained only from systemic sites. These data suggested that these isolates are potentially pathogenic. However, a larger number of isolates must be evaluated in order to confirm this hypothesis. Rapp-Gabrielson and Gabrielson[8]

also evaluated the relationship between serovar and site of isolation and reported that serovars 4 and 5 were frequently isolated from pigs with polyserositis, while serovar 2 was mainly isolated from pigs without polyserositis.

Evaluation of distribution of serovar-4 isolates (**Fig 1**) among herds revealed that this serovar was obtained from 40% of the evaluated herds. Furthermore, different herds were affected by different strains from serovar 4. Similar results were observed for herds affected by strains from serovars 2 and 12. These findings highlight an important epidemiologic feature of *H parasuis* infections among unrelated herds, which may have some implications in disease control. It is known that there is no broad-spectrum vaccine to control *H parasuis* infections and that results of commercial vaccine use are generally inconsistent among herds. These results may be associated with lack of cross-protection among *H parasuis* strains, even within the same serovar group. Although there is evidence for the lack of cross-protection among different stains from the same serovar group,[6, 7] further studies are necessary to evaluate the implications of genetic variability in cross-protection.

Most *H parasuis* strains with similar genotypes were included in the same serovar group, which indicated that the genotype of an isolate is a good predictor of its serovar group. However, some exceptions were observed, particularly in the group of isolates classified as serovar 1. In this group, strains with similar genomic fingerprints were either classified as serovars 1 or 13, or as NT. Similar results were observed for other isolates with similar genotypes and different serovars (**Fig 1**). The lack of association between genotype and serovar in this group of strains may be attributable to several factors. The serovar group of some of these strains may not have been correctly identified by AGPT. The AGPT involves a subjective interpretation of the presence or absence of an identity line between the specific antiserum and the extracted heat-stable antigens. In addition, some of the strains may not produce sufficient antigens to be detected by the test. It is known that serial passages in vitro may allow the selection of non-capsulated clones of *H parasuis*.[8] Another consideration is the nature of the repetitive sequences that were used as targets for the production of genomic fingerprints. These genomic regions are non-coding sequences located between active genes. The outcome of the rep-PCR reaction is the amplification of the sequences located between the repetitive sequences.[17] Most of the recombination events that occur in the bacterial genome involve the participation of repetitive sequences, since these are unstable regions in the DNA.[22] With such conditions, it would be possible to have 2 bacterial strains with similar genomic fingerprints but different serovar antigens.

To evaluate the molecular epidemiology of *H parasuis* within systems, individual dendrograms for multi-farm systems with $\geq$ 10 isolates were constructed. Results indicated that disease caused by *H parasuis* in a specific multi-farm system was associated with the presence of 1 (system D), 2 (system G), or 3 (system C) prevalent strains. Systems C and D, which had the same source of animals, had 1 of the prevalent *H parasuis* strains involved in disease in common, while system G had genetically different prevalent strains. These data corroborated findings of other studies in which only a few prevalent *H parasuis* strains were involved in disease in a specific herd. By means of REF analysis, Smart et al[13] evaluated the occurrence and distribution of various strains of *H parasuis* in swine herds from Ontario. In most herds, several strains of *H parasuis* could be detected although 1 or 2 strains usually predominated. Comparison of strains isolated from specific pathogen-free and conventional herds revealed that these herds were affected by different *H parasuis* strains, which suggested that the source of pigs may influence the herd distribution of pathogenic strains.[13]

Evaluation of the clonal relationship between strains from respiratory tract and systemic sites revealed that *H parasuis* isolates obtained from the upper respiratory tract differed considerably from strains isolated from lungs of pigs with pneumonia and from systemic sites such as pleura, pericardium, peritoneum, joints, and brain. In other studies,[13,23] *H parasuis* isolates obtained from nasal and systemic sites in healthy and clinically affected animals had distinct REF patterns. Our data indicated that there was no major difference between *H parasuis* strains isolated from lungs of pigs with pneumonia and systemic sites. These isolates were evenly distributed throughout the dendrogram that grouped the 98 *H parasuis* isolates and the genetic diversity of the strains obtained from these 2 sources was similar (20 strains were obtained from lungs of pigs with pneumonia and 22 were isolated from systemic sites). These results differ from those reported by Ruiz et al[4]; those investigators evaluated the clonal relationship between *H parasuis* strains obtained from respiratory tract and systemic sites and found that respiratory tract isolates had higher genetic diversity compared with isolates from systemic sites. This discrepancy may be the result of several factors. The numbers of *H parasuis* isolates characterized differed considerably between studies. In our study, 98 *H parasuis* isolates were characterized and compared, while Ruiz et al[4] evaluated 53 isolates. Furthermore, genomic fingerprints were compared with computer software in our study, which facilitated the identification of clusters of strains; *H parasuis* isolates genotyped by Ruiz et al[4] were assigned to groups by visual inspection. Both studies examined *H parasuis* isolates obtained from the VDL at the University of Minnesota, which introduces bias associated with sample submission by field veterinarians.

Results obtained in our study provided important information regarding the molecular epidemiologic features of *H parasuis* field isolates recovered from US herds (including herds from Illinois, Kansas, Kentucky, Minnesota, Missouri, Oklahoma, Texas, Wisconsin, and Wyoming). Isolates from a specific serovar group included strains with distinct genomic fingerprints. Furthermore, it was observed that most of the evaluated herds were affected by different strains from the same serovar group. These results raised the question of whether distinct strains from the same serovar group can induce satisfactory cross-protection. The association between genotype and protection remains to be defined. A high percentage (67%) of the herds were affected by NT strains. Because accurate characterization of *H parasuis* isolates that cause disease in a specific herd is essential for disease control, we believe that serotyping of isolates provides limited information compared with that obtained via genotyping, particularly with regard to selection of strains for inclusion in autogenous vaccines.

Lay Interpretation: The characterization of *Haemophilus parasuis* strains is very important for the study of the epidemiology of this agent within and between herds. In the present study, the characterization of *H. parasuis* field isolates by serotyping and genotyping was compared, and the molecular epidemiology of this agent in North American herds was studied. Results showed that genotyping provided a more accurate characterization of *H. parasuis* isolates, allowing the comparison of any isolate regardless of its serovar. Serotyping proved to be a limited technique for epidemiological studies on *H. parasuis* infections. A high genetic diversity was observed within serovars groups. It was observed that affected herds had 1 to 3 prevalent strains of *H. parasuis*, and that although many herds were affected by the same serovar group, each herd had different prevalent strains. It was also demonstrated that herds receiving the same source of animals shared similar prevalent strains. The epidemiological data presented in this studied can be further used for the development of control strategies to reduce mortality in swine herds due to *H. parasuis* infections.

Resources:

[a]PrepMan Ultra Sample Preparation Reagent, Applied Biosystems, Foster City, Calif.
[b]PCR reagents, Roche Molecular Diagnostics, Indianapolis, Ind.
[c]GeneAmp PCR System 2400, Applied Biosystems, Foster City, Calif.
[d]HiLo DNA Marker, Minnesota Molecular, Minneapolis, Minn.
[e]BioNumerics Version 2.5, Applied Maths, Kortrijk, Belgium.

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Table 1—Number, serovars, and strains of *Haemophilus parasuis* isolates obtained from 15 US swine herds and systems (A to O).

Herd or system	Location	No. of isolates	Serovar groups	Strain groups
Herd A	MN	6	3, 14, NT	4, 18, 31, 32
Herd B	MN	1	12	18
System C	MN, OK, KS	43	2, 7, 4, 12, 14, 3, 1, 13, NT	5, 9, 10, 11, 12, 13, 14, 15, 17, 18, 19, 20, 21, 22, 25, 26, 28
System D	IL, OK, WI	11	2, 4, NT	2, 8, 10, 11, 12, 16, 20, 22, 29
Herd E	MN	2	5, 14	18, 22
System F	MN	2	5, NT	1, 18
System G	TX	12	4, 12, NT	19, 22, 23, 24, 27
Herd H	MN	2	7, NT	6, 30
System I	MO	4	4, NT	3, 31
Herd J	IL	1	2	4
Herd K	KY	1	NT	29
Herd L	IL	1	4	15
Herd M	WY	2	2, 4	7, 16
Herd N	MN	3	NT	16, 20
Herd O	MN	7	1, 3, NT	28, 31, 32, 33

Table 2– Serovar distribution of 89 *Haemophilus parasuis* field isolates according to the isolation site.

	Serovar										
	1	**2**	**3**	**4**	**5**	**7**	**12**	**13**	**14**	**NT**	**Total**
Tonsil/ Nasal Cavity	1	0	6	0	0	0	0	0	0	5	12
Pneumonia	3	0	2	21	0	1	2	0	1	4	34
Systemic sites	3	3	0	15	1	0	4	1	1	15	43

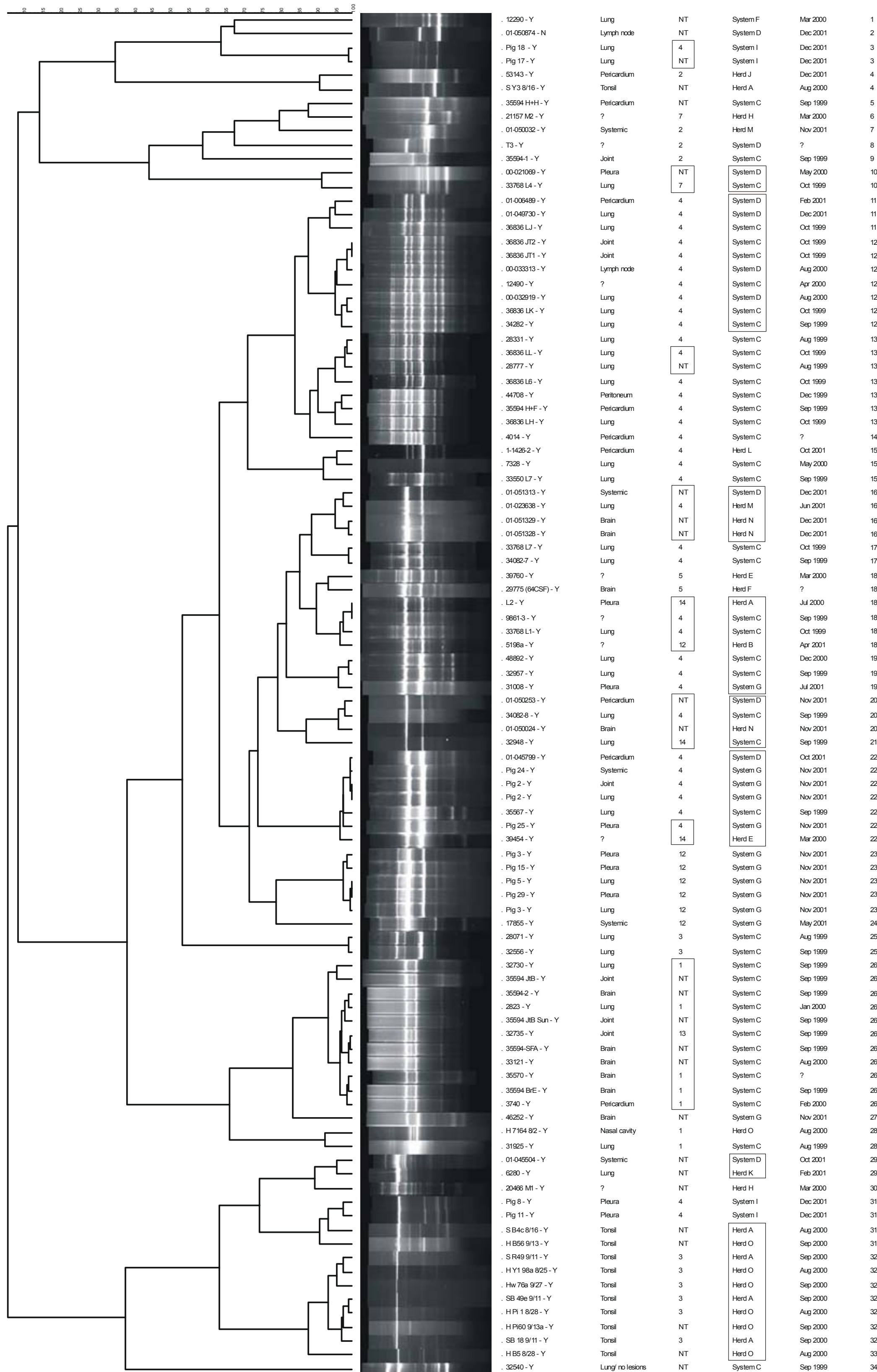

Figure 1—Cluster analysis by means of enterobacterial repetitive intergenic consensus-based PCR (ERIC-PCR) fingerprinting of 98 *Haemophilus parasuis* isolates obtained from 15 US swine herds and systems. The scale indicates the percentage of genetic similarity. Columns (left to right) indicate isolate identification, site of isolation, serotype, system or herd of origin, date of isolation, and strain group. Isolates were classified into 34 subtypes (strains) using a 90% similarity cutoff. In the serovar column, examples of strains with similar genotype and different serovars are boxed. In the herd column, examples of herds affected by similar *H parasuis* strains are boxed.

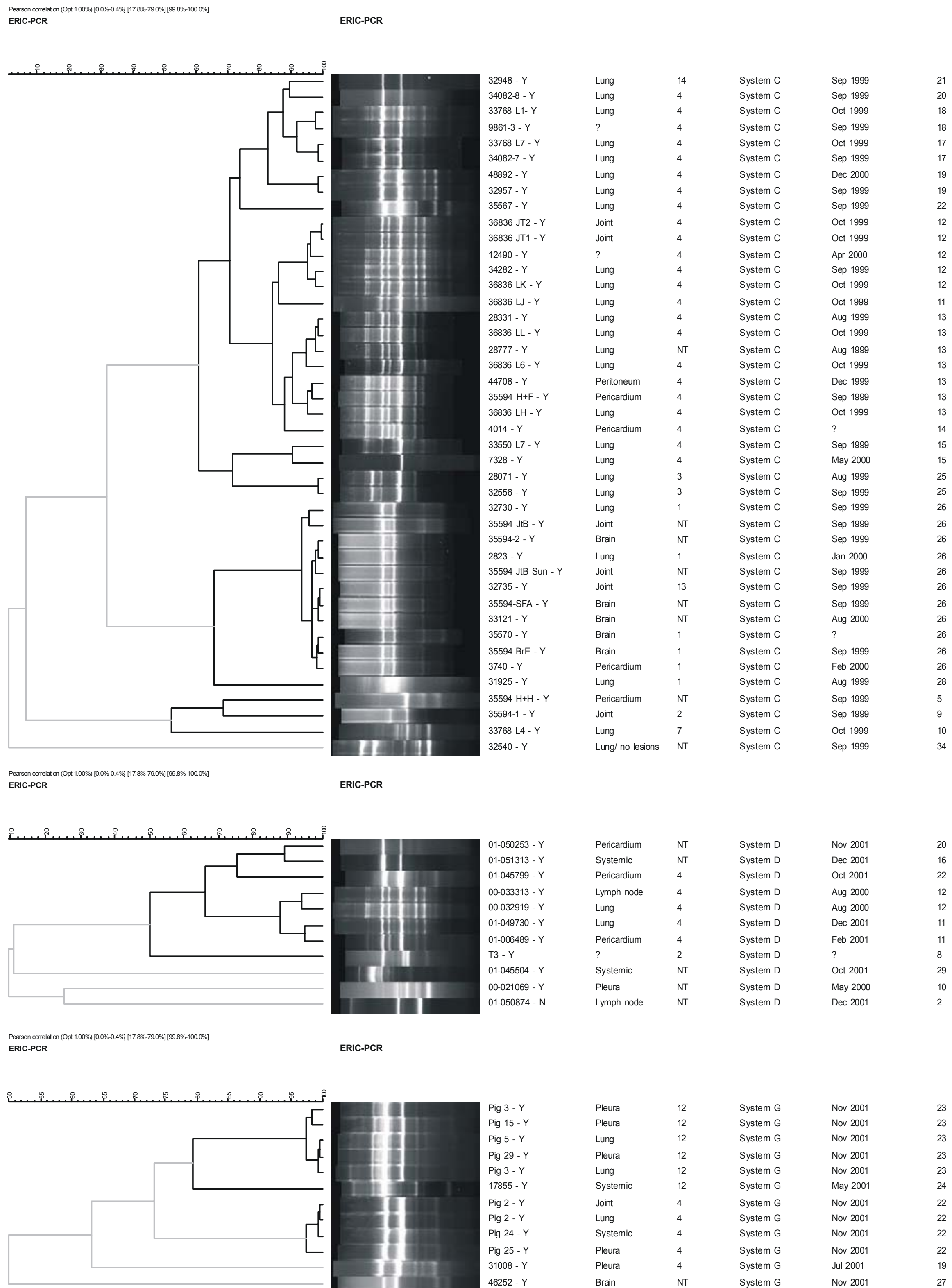

Figure 2— Cluster analysis by means of ERIC-PCR fingerprinting of *H parasuis* field isolates recovered from 3 US swine systems (C, D, and G). The scale indicates the percentage of genetic similarity. Columns indicate isolate identification, site of isolation, serotype, system of origin, date of isolation, and strain group. Significantly different clusters of strains are shown in pale lines. Clusters were defined based on the calculation of the point-bisectional correlation (BioNumerics).