RESEARCHREPORT
SWINE HEALTH

Title: Demonstration of pathogenicity in CDCD pigs with a cytopathic agent isolated from farm with "Sow abortion and mortality syndrome"
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Abstract:

A previously unidentified cytopathic agent along with PRRS virus was isolated from farms with history of severe abortion and sow mortality. Pathogenic role of the agent was not known in pigs. The objective was therefore to investigate clinical disease in cesarian derived colostrum deprived (CDCD) pigs following experimental inoculation with the agent. Molecular and serologic characteristics of the agent were also examined. The agent was serologically related with *Mycoplasma hyorhinis*, while the agent did not grow in any of mycoplasma media. Nucleotide sequence analyses showed 91.9% similarity with 16S rRNA of *M. hyorhinis*. In order to examine the agent in samples, PCR primers that can differentiate from *M. hyorhinis* were developed. Twenty CDCD and eight conventional 3-week old pigs were inoculated with the agent in different routes with or without presence of PRRS virus infection. None of the pigs showed clinical sign or gross pathological lesion, although evidence of the infection was detected by PCR. In conclusions, the agent is characterized as a variant of *M. hyorhinis* but not pathogenic to the pigs.

Introduction

During late 1996, an acute abortion problem has been reported from midwestern U.S. swine farms. Main clinical signs were high mortality (>5%) and abortions (>10%) in sows of all parities and all stages of gestation. These syndromes were confused with those of classical porcine reproductive and respiratory syndrome (PRRS). Because of these unusual clinical signs, the disease was initially named sow abortion and mortality syndrome (SAMS). The syndrome was also called atypical, acute or severe PRRS, because PRRS virus has been routinely isolated from the samples of the affected farms. Possible causes suggested to explain the clinical outbreaks were infection with a more virulent form of PRRS virus, PRRS virus in combination with other agent, or with a

previously unrecognized agent. While SAMS is known to be caused by PRRS virus variants, a previously unidentified cytopathic agent (named SAMS agent) has been isolated from samples from farms with a history of SAMS. Pathogenicity of the agent in pigs was not known.

Objectives

Immediate objective was to compare clinical signs and pathological lesions in CDCD pigs following inoculation of the SAMS agent with or without PRRS virus. This project was aimed at determining any pathogenic role of the SAMS agent in disease free CDCD pigs. In addition, attempts were made to characterize the agent by serologic and molecular methods.

Procedures

Experimental design - Twenty 3-week old CDCD pigs were purchased from Struve Labs, Manning, IA and inoculated with SAMS agent with or without PRRS virus as shown in Table 1. A swine testicle (ST) cell culture grown SAMS agent (12^{th} passage, ATCC VR-2580) was inoculated intranasally, intratracheally or intraveneously (IV) with or without additional PRRS virus inoculation. The PRRS virus used was from a farm with history of severe abortion (obtained from Dr. Swanson, NVSL, Ames, IA). The isolate is believed to be a hot strain of PRRS virus. Following inoculation of each pig, clinical signs were observed daily, and serum samples were collected weekly. The pigs were killed at different days post-inoculation (pi), and tissue samples were collected. Serum and tissue samples have been stored at -80 C for examination of SAMS agent and PRRS virus.

In order to examine pathogenicity of a low passage SAMS, three 3-week old pigs each from a high health farm were inoculated with a third passage SAMS IV or intraperitoneally (IP) along with 2 control pigs. One pig each inoculated IV or IP was killed at 7 days pi, and the remaining 2 pigs each were killed 14 days pi. Attempts were made to recover the agent from inoculated pigs by isolation and PCR assays.

Cultural, serologic and molecular characterization – Characteristics of the SAMS agent were examined in comparison with a laboratory adapted strain (GDL) of *M, hyorhinis* (obtained from Dr. Kim Wise at the University of Missouri). The SAMS agent and GDL strain were inoculated into tubes containing one of 3 referenced *M. hyorhinis* broth media: Friis medium containing no antibiotics, beef heart infusion (BHI) broth and Hayflick's broth. The tubes were incubated at 37 C, and evidence of the growth was observed daily for one month. Centrifuge concentrated SAMS and GDL were coated on nitrocellulose membrane strips, and dot blot assays were performed using mycoplasma reference sera. In addition, ST cell cultures infected with SAMS or GDL were reacted with the reference sera, and specific fluorescence was examined.

Nucleotide sequence similarity of SAMS agent were examined among 16S rRNA genes of swine mycoplasmas. Sequence pair distances were generated using Clustal method with Weighted residue weight table in MegAlign software. PCR primers specific for *Mycoplasma hyorhinis* or specific for SAMS agent were synthesized at the DNA Core Facility, University of Minnesota, St. Paul, MN. The selection of primers for PCR amplification was based on previously published data.

Isolation of SAMS agent by cell culture and PCR - Sera and tissues from inoculated pigs were examined by inoculating the samples onto ST cell monolayers. CPE was observed daily for 5-7 days. Samples with negative CPE were passaged blindly two more times. The DNA from sera or tissues was extracted using the Dneasy™ tissue kit (Qiagen Inc, Valencia, CA) according to the manufacturer's instructions. PCR was performed by a routine method. PCR products were resolved by elctrophoresis in a 1% agarose gel containing ethidium bromide.

Table 1. Experimental inoculation and necropsy examination of 3-week old CDCD pigs with SAMS agent and/or PRRS virus

Exp	No. of	Inoculation days			Pigs examined at days post-inoculation					
No.	pigs	0	7	42	0	7	17	31	42	56
A	3	S/IN					1	2		
B	3	S/IT				1	1		1	
C	2	S/IN		PRRS*						2
D	2	S/IV		PRRS						2
E	3	PRRS							1	2
F	3	PRRS	S/IV						1	2
G	4	None			1	1			2	

* PRRS virus was inoculated intranasally
S = SAMS agent; IN = intranasally; IT = intratracheally; IV = intravenously

Results

Clinical signs and gross lesion in CDCD and conventional pigs - None of the 3-week old CDCD pigs showed a significant clinical sign following inoculation of SAMS agent and/or PRRS virus throughout the experimental period. Following inoculation, temperature of SAMS inoculated pigs ranged between 99 and 103F. An increased temperature up to 104 F for 1-2 days was noted in PRRS virus infected pigs. No abnormal respiratory sign or swollen joint problem was observed. Similarly, there was no clinical sign in the 3-week old conventional pigs following inoculation with a low passage SAMS. At euthanisia, no abnormal gross lesion was observed in any of the pigs.

Detection of SAMS and PRRS virus - PCR primer sequences specific for *M. hyorhinis* or for SAMS agent are shown in Table 2. The use of primers specific for SAMS agent allowed detection of SAMS agent only, while primers specific for *M. hyorhinis* detected both *M. hyorhinis* and SAMS agent (Fig. 1). By using ST cell culture, SAMS agent was also isolated from sera or tissues of inoculated CDCD pigs, but the results were inconsistent. Some of the isolation results were confirmed by PCR using primers specific for SAMS agent. PRRS virus was readily recovered from all pigs following inoculation. PRRS virus viremia was evident in pigs for up to 34 days pi. PRRSV IFA antibody was first detected between 12-14 days pi, and high antibody titers were maintained until the end of experiment. SAMS agent was also detected by PCR from lung, liver and spleen of the 3-week old conventional pigs following inoculation.

Table 2. Oligonucleotide primers used to amplify the 16S rRNA gene sequence

Primer	Sequence (5' to 3')	
SAMA agent		
MHS –868	TTA GCT GCG TTA GTGAAA TTA C	Forward
MHS-1419	ATT ACC AAC TCC CAT GGT GC	Reverse
Mycoplasma hyorhinis		
MHA–868	TTA GCT GCG TTA GTGAAA TTA A	Forward
MHA-1419	ATT ACC AAC TCC CAT GGT GT	Reverse

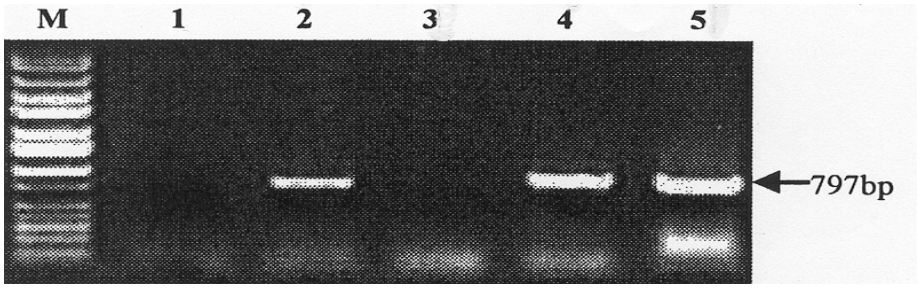

Fig. 1. Differential PCR using primers specific for *M. hyorhinis* or SAMS agent. Lane M – Hi-Lo DNA marker; Lane 1 – uninfected ST cells, Lanes 2 and 3 - *M. hyorhinis* ATCC strain; Lanes 4 and 5 – SAMS infected ST cell supernatant. *M. hyorhinis* specific primers were used for lanes 1, 2 and 4; SAMS specific primers were used for lanes 2 and 5. *M. hyorhinis* specific primers detected both M. hyorhinis and SAMS, but SAMS specific primers detected only SAMS.

Cultural, Serologic and molecular characterization – No growth of SAMS agent was detected in any of the 3 mycoplasma broth media following observation for one-month after inoculation, while the GDL strain grew readily in all three broth media. The dot blot assay showed that SAMS agent reacted positively with *M. hyorhini*s reference serum. Infected ST cell monolayers stained positive for *M. hyorhini*s 24 and 48 hours following infection with either SAMS or GDL strain. By sequence analysis, SAMS agent showed a high homology (91.9%) with *M. hyorhinis*. Since the SAMS agent is genetically different from *M. hyorhini,* a strain was deposited to American Type Culture Collection (ATCC VR 2580). Nucleotide sequences of the isolate VR-2580 16S rRNA and *M. hyorhinis* were also deposited to GenBank (accession nos. AF121890 and AF121891, respectively).

Table 3. Nucleotide sequence similarity among rRNA genes of swine mycoplasmas

	% similarity / % divergence			
16S rRNA (~870-bp)	SAMS	*M. hyorhinis*	*M.hyopneumoniae*	*M. flocculare*
SAMS	***			
M. hyorhinis	91.9 / 1.1	***		
M. hyopneumoniae	75.9 / 9.9	87.3 / 9.3	***	
M. flocculare	75.0 / 10.2	86.1 / 9.3	88.1 / 4.0	***

SAMS (VR-2580) 16S rRNA - AF121890; *M. hyorhinis* 16S rRNA - M24658; *M. hyopneumoniae* 16S rRNA - E02783; *M. flocculare* 16S rRNA - X62699.

In this study, characteristics of the agent were similar to those of *M. hyorhinis*, while the agent did not grow in any of mycoplasma media. Nucleotide sequences of 16S rRNA and 25S rRNA of the agent were determined, and PCR primers that can differentiate from *M. hyorhinis* were developed. However, none of the pigs showed clinical sign or gross pathological lesion, although evidence of the infection was detected by PCR. In conclusions, the agent is classified as a variant of *M. hyorhinis* but not pathogenic to the pigs.