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Case report

Identification of *Trueperella abortisuis* contamination in extended boar semen

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Summary

Trueperella abortisuis is a gram-positive bacterium that has been previously identified in aborted porcine feti and placentae located in Asia and Europe. Routine microbiological screening of extended boar semen from a US mid-Atlantic stud identified delayed growth of very small white colonies on both brain-heart infusion and sheep-blood agars after 48 to 72 hours incubation at 37°C under aerobic conditions. Isolate identification was performed using matrix-assisted laser desorption-ionization time of flight (MALDI-TOF) mass spectrometry, with *T abortisuis* identified (MALDI biotyper score = 2.103). After storage at 16°C and 72 hours post collection, total and progressive motility parameters had decreased in extended semen samples positive for *T abortisuis*. Further work is needed to elucidate the role *T abortisuis* may play in extended boar semen quality, extended semen longevity, and sow reproductive performance.

Keywords: swine, *Trueperella abortisuis*, boar, extended semen

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Resumen - Identificación de la contaminación con *Trueperella abortisuis* en semen diluido de cerdo

La *Trueperella abortisuis* es una bacteria gram-positiva que ha sido previamente identificada en fetos porcinos abortados y placentae en Asia y Europa. El monitoreo microbiológico rutinario de semen diluido en un centro de inseminación artificial en el medio del Atlántico de los Estados Unidos identificó el crecimiento retrasado de colonias blancas, muy pequeñas, tanto en agar de infusión de corazón-cerebro, y de sangre de oveja después de 48 a 72 horas de incubación a 37°C, bajo condiciones aeróbicas. Los aislamientos fueron identificados por espectrometría de masa asistida de laser utilizando el tiempo de vuelo desorción-ionización láser asistida por matriz (MALDI-TOF por sus siglas en inglés), identificando *T abortisuis* (MALDI puntaje de biotipo = 2.103). Después de su conservación a 16°C y 72 horas post recolección, los parámetros de motilidad progresiva y total disminuyeron en las muestras de semen diluido, positivas por *T abortisuis*. Es necesario más trabajo para esclarecer el papel que la *T abortisuis* pueda tener en la calidad del semen diluido, sobre la longevidad del semen, y el desempeño reproductivo de la hembra.

Résumé - Identification de *Trueperella abortisuis* dans de la semence de verrat diluée

Trueperella abortisuis est une bactérie à gram-positif qui a été identifiée préalablement en Asie et en Europe dans des fœtus avortés et des placentae de porc. L'examen microbiologique de routine de la semence de verrat diluée provenant d'un mâle reproducteur d'un état américain situé au milieu de la côte Atlantique a permis de mettre en évidence la croissance tardive de très petites colonies blanches sur des géloses d'infusion de cerveau et de cœur et des géloses supplémentées de sang de mouton après 48 à 72 heures d'incubation à 37°C dans des conditions aérobiques. Les isolats ont été identifiés par spectrométrie de masse du temps de vol suite à la désorption-ionisation par laser assistée d'une matrice (MALDI-TOF), et *T abortisuis* identifié (pointage du MALDI biotype = 2,103). Après entreposage à 16°C pendant 72 heures post collecte, les paramètres de mobilité totale et progressive avaient diminué dans les semences diluées positives pour *T abortisuis*. Des travaux additionnels sont requis afin d'élucider le rôle que pourrait jouer *T abortisuis* dans la qualité de la semence de porc diluée, sur la longévité de la semence diluée, et les performances de reproduction des truies.

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Bacteriosemina, the presence of bacteria in semen, is a common issue that needs to be controlled at artificial insemination centers in order to optimize extended semen quality and herd reproductive performance.[1] Sources of bacterial contamination of semen are varied and can be generally categorized as those originating from the animal (eg, feces, urogenital tract, preputial fluids, skin, hair, or respiratory secretions, or from personnel) and those of non-animal origin (eg, water sources, plant matter, ventilation systems, sinks and (or) drains, or laboratory material).[1] In the boar, bacteria belonging to the *Enterobacteriaceae*[1,2] and *Pseudomonaceae*[3] families appear to be the isolated contaminants that are most commonly identified in extended semen. To the knowledge of the authors, this report describes for the first time the presence in extended boar semen of *Trueperella abortisuis*, a bacterium that has been isolated from placentae of aborted sows and that has been suggested as an emerging pathogen in swine.

Isolation and identification of *T abortisuis*

A small mid-Atlantic US stud housing purebred adult boars (greater than 1 year of age) submitted extended cooled doses (Beltsville thawing solution [BTS] with gentamicin; IMV International, Maple Grove, Minnesota) to the Reference Andrology Laboratory of the University of Pennsylvania (Kennett Square, Pennsylvania) for routine spermiogram and microbiological analyses. Upon arrival (within 24 hours post collection and processing), subsamples were obtained and screened at 24, 48, and 72 hours using brain-heart infusion (BHI)

agar (BD Biosciences, Baltimore, Maryland) and sheep-blood agar (SBA; Remel, Kansas) to determine bacterial load and time-kill kinetics. All plates were incubated at 37°C in an aerobic atmosphere supplemented with 5% CO_2, with quantification of colony forming units (CFUs) performed using an illuminated plate reader after 24, 48, and 72 hours of incubation.

In an initial single-sire dose submission (Male 1), mixed growth was observed on both BHI and SBA plates from samples plated 24 and 48 hours post collection, with pure growth of a tiny pale colony observed after 72 hours of incubation. The remaining pure growth was re-plated on SBA and submitted to the Pennsylvania Animal Diagnostic Laboratory System (Kennett Square, Pennsylvania) for bacterial identification by matrix assisted laser desorption-ionization time of flight (MALDI-TOF) mass spectrometry using the MALDI Biotyper (Bruker Daltonics; Billerica, Massachusetts). Results identified the contaminant as *T abortisuis* (MALDI biotyper score = 2.103). A score cut-off ≥ 2.000 is recomended by the manufacturers for species-level detection.

Concurrent spermiogram results from the extended semen of Male 1 showed substantial decreases over time in total and progressive motilities, as determined using computer-assisted sperm analysis (HTM-IVOS; Beverly, Massachusetts). Sample total and progressive motilities at arrival were 90% and 67%, respectively. After the extended semen had been stored for 72 hours at 16°C, total and progressive motilities had dropped to 4% and 0%, respectively. *Trueperella abortisuis* was the only contaminant isolated from the low-motility samples.

Subsequently, BTS-extended semen from an additional four males (Male 2, Male 3, Male 4, and Male 5) standing at the same stud were purposely collected, processed, and submitted to the University of Pennsylvania Reference Andrology Laboratory for microbiological screening. After 48 hours post collection and processing, samples were plated on BHI and SBA with daily assessment of growth for up to 72 hours. Selected colonies were re-isolated on SBA, incubated under both aerobic and anaerobic conditions, and then identified using a Microflex LT MALDI-TOF Biotyper (Bruker Daltronics, Inc, Billerica, Massachusetts). Growth was observed under both culture conditions, with higher counts observed when cultures were incubated anaerobically (10^2 CFU per mL under aerobic conditions versus 10^3 to 10^4 CFU under anaerobic conditions) (Table 1). MALDI-TOF analysis demonstrated that *T abortisuis* was present in the extended semen of three of the four boars. The minimum inhibitory concentrations (MICs) (ARIS Sensititre; ThermoFisher Scientific, Waltham, Massachusetts) for selected antimicrobials were determined for the *T abortisuis* isolate (Table 2). Although this isolate was resistant to gentamicin, it was sensitive to the common beta-lactam antibiotics used in commercial porcine semen extenders.

Spermiogram analysis revealed similar changes in total and progressive motility scores over time. Average (± standard error of the mean [SEM]) total and progressive motilities of samples contaminated with *T abortisuis* (N = 3) at arrival were 86.3% ± 2.3% and 50.3% ± 14.4%, respectively. After the extended semen had been stored at 16°C for 72 hours, average (± SEM) total and progressive motilities had decreased to 6.3% ± 0.3% and 1.0% ± 0%, respectively.

Discussion

To the authors' knowledge, this is the first time that *T abortisuis* has been isolated from and identified in extended boar semen. Currently, literature concerning this bacterium is sparse. *Trueperella abortisuis*, previously known as *Arcanobacterium abortisuis* and reclassified to its current name in 2011,[4] is a gram-positive, diphtheroid-shaped organism that was first reported when isolated from a sow's aborted placenta in Japan by Azuma et al.[5] This first report led Ülbegi-Mohyla et al[6] to re-analyze strains suspected to be *Arcanobacterium abortisuis* isolated from the vagina, cervix, kidney, and urine of nine pigs between 1999 and 2007 by phenotypic properties and by sequencing the 16S-23S rDNA intergenic spacer region. Their results demonstrated that the strains were *Trueperella (Arcanobacterium) abortisuis*. Ülbegi-Mohyla et al[6] also reported that the bacterium was normally isolated with other microorganisms such as *Acinetobacter* species, *Branhamella* species, *Corynebacterium* species, *Enterococcus* species, *Escherichia coli*, *Flavobacterium* species, *Staphylococcus* species, or *Streptococcus* species. Similarly, in the current study, *T abortisuis* was present in an extended semen sample (Male 1) with mixed contaminants that included *Bacillus* species, *Corynebacterium* species, *Klebsiella* species, *Pseudomonas* species, *Staphylococcus* species, and *Streptococcus* species. More recently, European work (Metzner et al)[7] has suggested that *T abortisuis* may be an emerging pathogen, with the report describing the presence of the bacterium in umbilical and anal swabs from aborted feti and aborted placentae of swine. Of added interest is that *T abortisuis* does not appear to be swine specific, as it has also been identified in other livestock species.[8]

In this case, decreases in total and progressive motility parameters were observed in extended-cooled samples by 72 hours post collection. Typically, in non-contaminated extended semen samples, motility parameters

Table 1: Total bacterial counts and *Trueperella abortisuis* counts in extended boar ejaculates submitted for testing to the University of Pennsylvania Reference Andrology Laboratory (Kennett Square, Pennsylvania) after storage at 16°C for 0 and 72 hours

	Total bacterial counts (CFU/mL)		*T abortisuis* counts (CFU/mL)	
	0 hours	72 hours post collection	0 hours	72 hours post collection
Male 1	4.2×10^3	1.0×10^2	2×10^1	1.0×10^2
Male 2	2.9×10^3	2.0×10^2	1×10^2	1×10^2
Male 3	3.0×10^2	0	0	0
Male 4	2.9×10^3	1.1×10^3	6×10^2	1.4×10^3
Male 5	3.1×10^3	1.4×10^3	1.8×10^3	1.4×10^3

CFU = colony forming units.

Table 2: Antimicrobial sensitivity of a *Trueperella abortisuis* isolate from extended boar semen*

Antimicrobial	MIC (μg/mL)
Amikacin	8
Ampicillin	1
Azithromycin	> 4
Ceftazidime	16
Ceftiofur	≤ 0.25
Chloramphenicol	≤ 4
Chlortetracycline	> 8
Clindamycin	> 16
Danofloxacin	1
Doxycycline	4
Enrofloxacin	≤ 0.25
Erythromycin	> 8
Gentamicin	> 8
Imipenem	2
Neomycin	8
Oxacillin + 2% NaCl	4
Oxytetracycline	8
Penicillin	0.12
Ticarcillin	≤ 8
Ticarcillin/clavulanic acid	≤ 8
Trimethoprim/sulphamethoxazole	≤ 5
Tylosin	32

* Minimum inhibitory concentration (MIC) refers to the lowest concentration of drug that inhibited growth (ug/mL).

decrease on average 1% to 4% per day of storage.[9] Follow-up contact with the boar stud found that no subjective decreases in motility in their extended-cooled semen doses had been observed. However, upon further inquiry, it was found that extended semen was not normally held beyond 48 hours post collection and it was recommended to use the product within 1 to 2 days of collection. The stud reported no health or other issues described by farms using the extended semen.

In conclusion, this case supports that *T abortisuis* can be present in a non-clinically affected boar stud and can contaminate extended boar semen. This contaminant may cause disruptions to extended porcine semen (ie, total and progressive motilities) when held at typical storage conditions (16°C) for several days prior to use. Further work needs to be performed to elucidate the role *T abortisuis* may play in extended boar semen quality and sow reproductive performance.

Implications

- *T abortisuis* can be identified in extended semen originating from non-clinically affected boars used in artificial insemination programs.
- Under the conditions of this study, *T abortisuis* exhibits slow growth in extended porcine semen, with isolation best found in sampling older stored semen samples (> 48 hours) followed by a 72-hour incubation under aerobic or anaerobic conditions.
- Under the conditions of this study, decreased total and progressive sperm motilities may be found in extended semen contaminated with *T abortisuis* by 72 hours post processing.
- Identification of the source(s) of *T abortisuis* contamination is necessary in order to better mitigate its presence in extended porcine semen.

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Conflict of interest

None reported.

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Conversion tables

Weights and measures conversions

Common (US)	Metric	To convert	Multiply by
1 oz	28.35 g	oz to g	28.4
1 lb (16 oz)	453.59 g	lb to kg	0.45
2.2 lb	1 kg	kg to lb	2.2
1 in	2.54 cm	in to cm	2.54
0.39 in	1 cm	cm to in	0.39
1 ft (12 in)	0.31 m	ft to m	0.3
3.28 ft	1 m	m to ft	3.28
1 mi	1.6 km	mi to km	1.6
0.62 mi	1 km	km to mi	0.62
1 in^2	6.45 cm^2	in^2 to cm^2	6.45
0.16 in^2	1 cm^2	cm^2 to in^2	0.16
1 ft^2	0.09 m^2	ft^2 to m^2	0.09
10.76 ft^2	1 m^2	m^2 to ft^2	10.8
1 ft^3	0.03 m^3	ft^3 to m^3	0.03
35.3 ft^3	1 m^3	m^3 to ft^3	35
1 gal (128 fl oz)	3.8 L	gal to L	3.8
0.264 gal	1 L	L to gal	0.26
1 qt (32 fl oz)	946.36 mL	qt to L	0.95
33.815 fl oz	1 L	L to qt	1.1

Temperature equivalents (approx)

°F	°C
32	0
50	10
60	15.5
61	16
65	18.3
70	21.1
75	23.8
80	26.6
82	28
85	29.4
90	32.2
102	38.8
103	39.4
104	40.0
105	40.5
106	41.1
212	100

$^{\circ}F = (^{\circ}C \times 9/5) + 32$

$^{\circ}C = (^{\circ}F - 32) \times 5/9$

Conversion chart, kg to lb (approx)

Pig size	Lb	Kg
Birth	3.3-4.4	1.5-2.0
Weaning	7.7	3.5
	11	5
	22	10
Nursery	33	15
	44	20
	55	25
	66	30
Grower	99	45
	110	50
	132	60
Finisher	198	90
	220	100
	231	105
	242	110
	253	115
Sow	300	135
	661	300
Boar	794	360
	800	363

1 tonne = 1000 kg

1 ppm = 0.0001% = 1 mg/kg = 1 g/tonne

1 ppm = 1 mg/L