Placentitis and abortion in domestic pigs (*Sus scrofa domesticus*) associated with *Trueperella abortisuis* on US swine farms

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**Summary**

We document a case series of abortions and placentitis in domestic pigs from the Midwest United States where aerobic bacterial cultures consistently isolated *Trueperella abortisuis*. Cases were submitted between 2017-2020 to the Kansas State Veterinary Diagnostic Lab. Microscopically, there was supplicative placentitis with necrosis and intralesional, gram-positive coccobacilli. In all cases, molecular diagnostics were negative for major causes of abortion in pigs. This is the first known report of *T. abortisuis* isolated from swine abortions or placentitis in the United States.

**Keywords:** swine, *Trueperella abortisuis*, abortion, bacterial placentitis.

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**Resumen - Placentitis y aborto en cerdos domésticos (*Sus scrofa domesticus*) asociados con *Trueperella abortisuis* en granjas porcinas de Estados Unidos**

Documentamos una serie de casos de abortos y placentitis en cerdos domésticos del Medio Oeste de los Estados Unidos donde de los cultivos de bacterias aerobias se aislaron consistentemente a la *Trueperella abortisuis*. Los casos se enviaron entre 2017-2020 al Laboratorio de Diagnóstico Veterinario del Estado de Kansas. Microscópicamente, había placentitis supurativa con necrosis y cocobacilos grampositivos intralesionales. En todos los casos, los diagnósticos moleculares fueron negativos a las principales causas de aborto en cerdos. Este es el primer reporte conocido de *T. abortisuis* aislada de abortos porcinos o placentitis en los Estados Unidos.

**Keywords:** cerdos, *Trueperella abortisuis*, aborto, placentitis.

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**Résumé - Placentite et avortement chez des porcs domestiques (*Sus scrofa domesticus*) associés à *Trueperella abortisuis* dans des élevages porcins Américains**


**Keywords:** porcs, *Trueperella abortisuis*, avortement, placentite.

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Acetaria in the genus *Trueperella* (formerly *Arcanobacterium*) are aerobic, gram-positive, diphtheroid-type cocci, and incorporate five species capable of causing variable disease among humans and animals: *Trueperella pyogenes, Trueperella abortisuis, Trueperella bernardiae, Trueperella bialowiesiensis,* and *Trueperella bonsai.*

Of these, *T. abortisuis* has been implicated as an emerging abortigenic and causative agent of supplicative placentitis in swine in Japan, Scotland, and Spain and has been isolated from the semen of clinically healthy boars in the United States. This bacterium was first isolated in 2006 from a 6-month-old barrow from Japan with necrotizing, hemorrhagic splenitis and multiple organ failure. At discovery, the bacterium was classified as an unpublished *Arcanobacterium* species strain HJ57-14E, with a 99.7% similarity using 16S rDNA gene sequencing. In 2009, the bacterium was isolated from the placenta of a sow following abortion, and the classification *Arcanobacterium abortisuis* was proposed before reclassification of the genus to *Trueperella* in 2011. *T. abortisuis* has been isolated from aborted fetal tissues and fetal membranes in Europe and Asia, and isolated from boar semen in Spain and the United States. Additionally, *T. abortisuis* has also been isolated in cases of metritis and vaginitis in cows, and in companion animals including a feline with nephroliths and uroliths, an anal sac abscess in a dog, and a perianal abscess in a cat. However, the

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significance of \( T\) abortisuis and route of infection is unclear, especially in companion animals and in nonreproductive pathology.

The current report summarizes a case series of abortion in gilts and sows submitted from 3 separate production systems to the Kansas State Veterinary Diagnostic Laboratory (KSVDL) between September 2017 and May 2020 in which \( T\) abortisuis and other bacteria were isolated from samples of placenta, fetal stomach contents, or uterine fluid from affected sows through aerobic bacterial culture and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) analysis. Gross and microscopic lesions in this case series were frequently identified in the placenta and consisted of a necrotizing, suppurrative placenitis with variable amounts of gram-positive cocci, frequently arranged in small clusters or pairs, consistent with bacteria as the cause of abortion. The major purposes of this case series are to report the identification of \( T\) abortisuis in swine farms across the Midwest United States and discuss its role as a potential abortigenic bacteria.

**Animal care statement**

An animal care protocol was not necessary as only submitted laboratory specimens were used in these cases. Farms associated with case 1 were certified in Pork Quality Assurance Plus.

**Case description**

**Case 1**

The first set of cases were submitted to KSVDL from September 2017 through November 2017 from an approximately 5600 gilt-sow farm in Kansas. The farm was experiencing reproductive failure in gilts with a 12% average decrease in conception rate by 30 days of gestation compared to historical data from the farm and cohort farms of similar size, genetics, geographic location, and management practices. The farm had a prior history of Senecavirus A (SVA) infection in the herd. Abortions were reportedly occurring in pregnant gilts and sows between 24 to 70 days of gestation. Affected gilts and sows were not clinically ill but showed clinical signs of reproductive failure including repeating cycles, abortion, and suppurative vaginal discharge with or without expulsion of the fetuses.

Multiple sets of formalin-fixed and fresh samples of aborted fetuses, ligated uterine loops and sections of uterine tissue, nasal swabs, feces, kidney, pooled saliva, and serum from numerous sows were submitted within that time frame. Aborted fetuses were not mumified or excessively autolyzed. Testing was performed on samples as requested by the referring veterinarian and variably included necropsy, histopathology, aerobic and anaerobic bacterial cultures, real-time polymerase chain reaction (PCR), serology, and metagenomic next generation sequencing.

In all submissions, aerobic culture was performed on samples of fetal stomach fluid, uterine lavage fluid, uterine swabs, fetal intracavitary swabs, or placental membranes, as available. Aerobic bacterial culture was performed using blood agar (Tryptone Soy Agar with 5% sheep blood); MacConkey agar, or Columbia CNA with 5% sheep’s blood at 37°C (± 2°C) with 5% CO\(_2\). Samples were streaked onto half to one-third of agar plates, incubated 15 to 24 hours, and then interpreted following standard laboratory procedures. Isolates were identified using MALDI-TOF MS using MALDI-TOF MS software (Bruker Daltonik), with standard protein extracts. A MALDI-TOF score > 2.0 indicated species identification, a score of 1.7 to 1.9 indicated genus identification, and a score < 1.7 indicated no identification or unreliable identification. Trueperella abortisuis was consistently isolated in uterine lavages. Other less consistent aerobic and anaerobic isolates in uterine lavages were identified (Table 1). Semen samples were systematically cultured and yielded no growth of bacterial pathogens.

Formalin-fixed tissues were processed according to standard protocols at the KSVDL. All tissues were stained with hematoxylin and eosin; placental membranes, uterine tissue, or fetal viscera were additionally stained with Twort’s Gram stain. Microscopically, uterus from affected sows had moderate to severe fibrinous suppurative endometritis with moderately ectatic endometrial glands containing few neutrophils (Figure 1). The uterine lumen of some sows revealed small numbers of primarily gram-positive coccobacilli, with fewer gram-positive small rods and cocci, and aerobic culture of a fresh sample of this uterus isolated abundant \( T\) pyogenes (Table 1). Placentas from these sows had multifocal areas of trophoblast necrosis and mild fibrinosuppurative placentitis and variably sized colonies of frequently clustered or paired gram-positive coccobacilli (approximately 0.5-1.0 µm), fewer gram-positive cocci (approximately 0.7 µm) and small (approximately 0.5-1.5 µm in length) bacilli, and similarly sized gram-negative bacilli. Microscopically, lung from one fetus had moderate suppurative pneumonia. No bacteria were identified with special stains on this fetus.

Paired serum samples from multiple sows from this herd were analyzed to determine serum concentrations of immunoglobulin M (IgM) against *Leptospira* serovars Canicola, Pomona, Grippotyphosa, Icterohaemorrhagiae, Hardjo, and Bratislava using a commercially available quantitative sandwich IgM-specific enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer’s recommendations. Several sows had elevated titers for *L*. Icterohaemorrhagiae and *L*. Canicola. The *L*. Icterohaemorrhagiae serovar microscopic agglutination test (MAT) had 5 of 19 sows with titers above 1:800 (ranging from 1:1600 to 1:12800). The *L*. Canicola serovar MAT had 5 of 19 sows with titers above 1:800 (ranging from 1:1600 to 1:6400). Two weeks following these results, 5 of 18 sows had titers for *L*. Icterohaemorrhagiae above 1:800 (ranging from 1:1600 to 1:12800), with only one sample (No. 5195) overlapping from the original sample set, which had maintained a titer of 1:1600 and had reportedly aborted. *Leptospira* PCR with DNA extraction using standard laboratory protocol was negative on samples of pooled tissue and on individual uterine samples from several sows. Porcine circovirus (PCV) type 2 real-time PCR was negative on pooled samples of serum. Virus isolation was not successful. Because of the history of SVA on this farm, PCR testing for this pathogen was performed and was negative on uterine swabs and fetal samples. Porcine parvovirus (PPV) hemagglutination had 2 of 19 sows with titers which were above 1:256 (ranging from 1:1024 to 1:512) suggestive of exposure. Fecal samples were submitted for PCR to detect porcine epidemic diarrhea virus, *Lawsonia intracellularis*, and delta coronavirus and were all negative. Swine influenza virus matrix PCR was negative on samples of pooled oral fluid and nasal swabs. Tetracore real-time PCR for porcine reproductive and respiratory syndrome virus (PRRSV) and *Brucella* buffered acid plate antigen (BAPA) were negative on samples of pooled serum. Pseudorabies glycoprotein B antibody testing was negative. *Mycoplasma*
Table 1: Bacteria isolates identified from samples submitted by 3 Midwest US swine farms experiencing increased abortions

<table>
<thead>
<tr>
<th>Case number and date</th>
<th>Submitted tissues</th>
<th>Gross lesions</th>
<th>Microscopic lesions</th>
<th>Bacteriology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 1; November 2017</td>
<td>Uterine fluid*</td>
<td>NA</td>
<td>NA</td>
<td>Abundant Trueperella abortisuis isolated from uterine fluid, Trueperella pyogenes, Pasteurella multocida, Streptococcus dysgalactiae, Enterococcus gallinarum, Escherichia coli (non-hemolytic and hemolytic), Proteus mirabilis, Streptococcus suis, Streptococcus species (beta-hemolytic), and Clostridium perfringens</td>
</tr>
<tr>
<td>Case 1; November 2017</td>
<td>Uterine fluid*</td>
<td>NA</td>
<td>T abortisuis isolated from uterine fluid, Actinobacillus rossii, E coli (non-hemolytic), Streptococcus hyointestinalis, Aerococcus viridans, gram-negative cocci (unable to identify), Acinetobacter lwoffii, Lactococcus raffinolactis, Aeromonas bestiarum, Streptococcus parauberis, Acinetobacter johnsonii, and C perfringens</td>
<td></td>
</tr>
<tr>
<td>Case 2; December 2017</td>
<td>Five aborted fetuses† and five placentas</td>
<td>Mild-moderate placental thickening and hemorrhage; pleural effusion and subcutaneous hemorrhage</td>
<td>Suppurative placentitis and intra-trophoblast gram-positive bacteria; hepatic congestion and renal hemorrhage</td>
<td>Abundant T abortisuis isolated from stomach contents, A rossii, E coli (non-hemolytic), S hyointestinalis, A viridans, gram-negative cocci (unable to identify), A lwoffii, L raffinolactis, A bestiarum, S parauberis, A johnsonii, and C perfringens</td>
</tr>
<tr>
<td>Case 2; December 2017</td>
<td>One aborted fetus‡ and one placenta</td>
<td>Diffuse placental thickening, multifocal tan-brown discolorations and roughening</td>
<td>Suppurative necrotizing placentitis, trophoblast sloughing, gram-positive and negative coccobacilli in airways and placenta</td>
<td>Abundant T abortisuis isolated from a fetal swab, E coli (non-hemolytic), Citrobacter gilleni, Aeromonas species, Lactococcus garvieae, Enterococcus faecium, A viridans, Enterococcus hirae, gram-negative cocci (unable to identify), S suis, Streptococcus species (Alpha hemolytic), Staphylococcus chromogenes, Streptococcus alactolyticus, S parauberis, L raffinolactis, Lactobacillus ruminis, Enterococcus hirae, and C perfringens</td>
</tr>
<tr>
<td>Case 3; May 2020</td>
<td>Three aborted fetuses§ and one placenta</td>
<td>Moderate amount of tan-yellow exudate covering allantois; umbilical cord and allantois hemorrhage</td>
<td>Suppurative gram-negative and gram-positive bacterial placentitis; suppurative, fibrinous omphalitis</td>
<td>Abundant T abortisuis isolated from placenta, E coli (non-hemolytic), and A viridans</td>
</tr>
</tbody>
</table>

* Unknown parity, age, or health status.
† Crown-rump length of fetuses 150-180 mm (approximately 60-70 days of gestation).
‡ Crown to rump length of fetus 70 mm (approximately 40-50 days of gestation).
§ Crown-rump length of fetuses 50-60 mm (approximately 40 days of gestation).
NA = not available or analyzed.
ELISA was positive or suspect in 17 of 22 (77.3%) submitted cases. The PCV type 2 quantitative indirect fluorescent antibody assay had 11 of 19 sows with titers above 1:320 (with a range of 1:640 to ≥1:5120). Trace mineral levels (including selenium) and vitamins A, D, and E were measured in serum of affected sows and were within normal limits for the dams. Metagenomic sequencing on a single uterus sample did not identify T. abortisuis or viral pathogens within the sample. Bacterial cultures were also done on this sample and did not yield T. abortisuis or other bacteria.

Case 2
In December 2017, groups of aborted fetuses and placentas from 11 sows were submitted to KSVDL from an 8000 gilt-sow farm in Wyoming with a reported history of worsening abortion rates year-to-year and a fetal loss rate of approximately 6% among pregnant gilts and sows at the time of submission. Submitted fetuses were between 35 and 95 days of gestation and the degree of post-mortem autolysis varied from mild to marked between groups. One fetal group was submitted without placental tissues. The 10 groups submitted with placental tissues had at least one sample of fetal membranes or placenta with a chorion or allantois which was multifocally to diffusely thickened, edematous, discolored grey to brown, or hemorrhagic. Microscopically, 6 of 10 groups (60%) with submitted placental tissue had lesions consistent with placental necrosis affecting up to 20% of the tissue. Of those, 3 of 6 (50%) had a fibrinosuppurative placentitis composed of moderate to abundant numbers of gram-positive coccobacilli, diplococci, and short gram-negative rods mixed with exfoliated trophoblasts. One fetus also had mats of moderate to abundant numbers of gram-positive coccobacilli mixed with fewer small, gram-positive rods and rare degenerate neutrophils or foamy macrophages within airways. Fetal swabs or stomach contents from all groups were submitted for bacterial culture, and two of the groups with gross and microscopically supplicative bacterial placentitis and pneumonia had abundant T. abortisuis isolated. Other aerobic and anaerobic isolates are listed in Table 1. Pooled samples of placenta and lung were submitted from multiple groups for real time PCR for PRRSV and PCV type 2 and type 3, which were all negative. Pooled samples of placenta submitted for PPV PCR were also negative. Metagenomic sequencing was performed on pooled samples of placenta and recovered approximately 46% eukaryotic (host genome), 42% bacteria, 3% virus (bacterial phage), and 6% other. A 550bp partial T. abortisuis 16S rRNA sequence was extracted from the sample reads and was 98.8% similar to T. abortisuis strain 15TRD1120-003 (MH040922).

Case 3
In May 2020, a placenta and three pig fetuses from one gilt were submitted for necropsy with additional testing from a 650 gilt-sow farm in Nebraska. History of abortion from other dams or maternal illness on the farm was not disclosed. Submitted fetuses had a gestational age of approximately 40 to 45 days and were randomly assigned identification A, B, or C. The fetuses and placenta were in good to fair postmortem condition. Fetus A was grossly unremarkable. Fetus B was contained within an amniotic sac covered in multifocal to coalescing, tan-yellow, purulent exudate and the allantois had a discrete, locally extensive area of green-brown discoloration. Fetus C was also contained within an amniotic sac and placenta corresponding to the umbilical cord and allantois was transmurally discolored red-black. Microscopically, submitted placenta had a fibrinosuppurative and necrotizing placentitis with few to moderate numbers of gram-positive coccobacilli, fewer cocci, and gram-negative, small-to-medium sized rods adhered to the trophoblast lining or adhered to sloughed, necrotic trophoblasts. Microscopically, fetus C had a suppurative, fibrinous omphalitis with similar intralational gram-positive coccobacilli and gram-negative small-to-medium rods. A sample of affected amnion from fetus B was submitted for aerobic bacterial culture and isolated...
abundant *T. abortisuis*, non-hemolytic *Escherichia coli*, and *Aerococcus viridans* (Table 1). Pool samples of heart, liver, lung, kidney, and spleen were submitted for PCR for PRRSV, PCV type 2 and type 3, and PPV which were all negative.

**Discussion**

There is a growing body of literature to support the potential role of *T. abortisuis* as an emerging abortigenic bacterium of swine. This bacterium has been previously isolated from the placenta, uterus, or fetus from clinically affected sows in Japan and some European countries, and from the semen of clinically healthy boars in the United States.2-4 To the authors’ knowledge, this is the first report of isolation of *T. abortisuis* from fetal tissues, placenta, and uterine samples in swine abortions in the United States. In our case series, abortion was not linked with any of the common porcine abortigenic etiologies. Common bacterial causes of abortion, including *Brucella suis* and *Leptospira*, were ruled out by negative ancillary testing as well as lack of typical clinical signs in the dam, which include fever, anorexia, icterus, abortion of fetuses near or full-term, fetal mummification, stillbirth, or birth of weak piglets that die shortly after birth.10-11 Porcine viral causes of abortion, including PRRSV, PCV type 2 and type 3, and PPV, and other viral etiologies were also ruled out by molecular analysis of fetal tissues and placenta, including metagenomics analysis in some cases. Gross and microscopic lesions consistently observed in placenta, uterus, and, in some cases, fetal lung in our case series were indicative of a bacterial etiology and were consistent with those described in previous reports in which *T. abortisuis* was isolated.3,6

The role of *T. abortisuis* in cases of endometritis and abortions in pigs has not been fully established to date.12 However, some previous reports have implicated its potential pathogenicity in abortion and reproductive failure in other countries.4,12 Some authors have reported isolation of *T. abortisuis* along with other bacteria in samples from nonclinical pigs suggesting that *T. abortisuis* could be a commensal (or opportunistic) pathogen of the urogenital tract of male and female pigs.2,13 As in the present case series, previous reports mention isolation of *T. abortisuis* along with a mixed bacterial population from affected tissues.2,13 But none of these other bacteria were consistently isolated in these cases. Most of the additional bacteria isolated in this case series have not been implicated as causative agents of abortion in porcine species, and to our knowledge are known skin commensals or contaminants from nonsterile tissue collection, as suggested in previous reports.12-14 In this present report, *T. abortisuis* was consistently isolated from most of the affected tissues in which gram-positive cocobacilli were observed on microscopic examination. This could suggest a potential role of *T. abortisuis* in porcine abortion and reproductive failure, whether it is as a primary pathogen or as a cofactor in association with other pathogens. Further research should focus on identifying pathogenic traits of *T. abortisuis*, its interaction with other commensal reproductive tract bacteria, and disease reproducibility. Surveillance and diagnostic testing to isolate and confirm the pathogenicity of *T. abortisuis* should continue.

Unlike other production animals, abortions and fetal loss in swine are usually due to viral infection, and abortions due to bacteria are often sporadic and of limited herd health significance, with reportedly less than 25% of abortion in swine due to bacteria.10,15 The route of infection in the cases presented in this report was not determined. In general, the pathogenesis of bacterial-induced abortion includes pre-existing metritis, ascending infection through the cervix, infection of the placenta or fetus following bacteremia of the dam, or maternal illness.15 In the submitted cases, ascending infection, subclinical metritis, or fetoplacental infection due to subclinical bacteremia are less likely, as dams were reportedly not showing any signs of systemic illness prior to abortion. Other potential sources of bacterial infection include semen, insemination tools or techniques, and fomites in the environment. *Trueperella abortisuis* has been isolated from testes of normal boars suggesting the bacteria could be commensal in the organ and could be a plausible source of infection.2 In case 1, extensive investigation included systematic aero- cultures from semen samples and sampling of the facilities and yielded no growth of bacteria. Several management changes were also implemented simultaneously on this farm, as the management team was uncertain if *T. abortisuis* was the primary pathogen given the relative lack of literature indicating its role as a primary abortigenic bacteria at the time of the isolation. These changes included transition from post cervical artificial insemination (AI) to traditional AI, emphasis of hygienic AI, increased barn ventilation, decreased barn humidity, and culling of sows/gilts returning to estrus with a purulent vaginal discharge. These protocols resulted in termination of cases of abortion. An inciting cause for immunosuppression which could have predisposed the gilts/sows to bacterial infection was not identified; biosecurity, sanitation, and insemination protocols were not disclosed on the farms from cases 2 and 3. Nutritional status and levels of vitamins and minerals, including selenium and vitamins A, D, and E were within normal limits in all affected animals within one farm in this case series.

In this case series, we also highlight the importance of submitting full sets of tissues, including fetal, placenta, and uterine samples (be it as uterine tissue or swabs from uterus), as fetal gross and microscopic lesions might be absent or non-specific in abortions. Aerobic and anaerobic bacterial culture results must be interpreted with caution in the absence of microscopic inflammatory lesions, as postmortem overgrowth and fecal or environmental contamination can result in bacterial isolates which may be irrelevant to the cause of abortion. Determining a definitive diagnosis for fetal death or abortion in production animals can be challenging given the numerous infectious and noninfectious potential causes that can contribute to fetal or embryonic loss. Isolation of infectious etiologies of abortion is dependent on appropriate and timely collection of aborted fetuses and placenta, and proper interpretation of diagnostic results. The placenta is often contaminated, and the best sample for bacterial isolation is stomach fluid from the aborted fetus or a swab of the pleural or peritoneal cavity of the fetus, which is not always available in submitted samples.10 To further complicate reaching a definitive diagnosis, all fetuses in a litter are not usually infected at the time abortion occurs, and fetuses may die or become infected at different points, which was also observed in these cases.15 Sporadic abortions are expected in large production operations, so many instances of fetal loss are never submitted for diagnostic evaluation. For these reasons, many causes of fetal death and abortion remain idiopathic. Submissions may also fail to include fresh tissue samples or inappropriately sized samples. In pigs, when gross or microscopic evidence of suppurative placentalitis or fetal
bronchopneumonia is identified, a bacterial cause for abortion should be included as a potential differential. While typically sporadic, bacterial causes of abortion in swine can contribute to economic losses especially when multiple abortigenic bacteria are isolated. *Trueperella abortisuis* as a sole or contributing cause of abortion in pigs has not yet been fully established but should be considered as a possible cause of bacterial abortion. All or some of the points discussed above could have played a role in the large gap of time of isolation between different cases.

In conclusion, this report brings attention to the isolation of *T. abortisuis* within a series of swine abortion cases and underscores the importance of an extensive diagnostic workup in cases of swine abortion to help rule in or out most common infectious causes of abortion.

**Implications**
- *Trueperella abortisuis* was isolated from swine abortions in the United States.
- No other viral or bacterial etiology was isolated.
- The role of *T. abortisuis* in swine abortions remains unknown.

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**Conflict of interest**
None reported.

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* Non-refereed reference.