

Clostridium novyi infection causing sow mortality in an Iberian pig herd raised in an outdoor rearing system in Spain

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Summary

Clostridium novyi was the suspected cause of death of two mature gestating Iberian-breed sows, on evidence of a gas-filled necrotic liver, rapid decomposition and tympany of the carcasses, and the absence of any other detectable cause of death. Anaerobic cultures yielded large numbers of *Clostridium*-like organisms, and *C novyi* type B was identified using a multiplex polymerase chain reaction (PCR) assay. In cases of unexpected mortality in gestating sows, veterinarians need to be aware of the most common causes of death, including *C novyi* infection. In order to achieve a correct diagnosis, it is essential to perform a postmortem examination and collect samples as soon as possible after death. In addition, use of PCR procedures may allow rapid identification of *C novyi* and the types implicated.

Keywords: swine, *Clostridium novyi*, Iberian breed pig, sudden death.

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Resumen - Infección por *Clostridium novyi* causa mortalidad en hembras en un hato de cerdos Ibéricos criado en producción extensiva en España

El *Clostridium novyi* fue la causa sospechada de muerte de dos hembras gestantes de raza Ibérica, basándose en la evidencia de hígado necrótico lleno de gas, una rápida descomposición y timpanismo de las canales, así como la ausencia de otra causa detectable de muerte. Los cultivos anaeróbicos rindieron grandes cantidades de organismos similares a *Clostridium*, y se identificó el *C novyi* tipo B utilizando una prueba multiplex de reacción en cadena de la polimerasa (PCR por sus siglas en inglés). En casos de mortalidad inesperada en hembras gestantes, los veterinarios deben estar conscientes de las causas más comunes de muerte, incluyendo la infección por *C novyi*. Para lograr un diagnóstico correcto, es esencial realizar un examen post mortem y coleccionar muestras después de la muerte tan pronto como sea posible. Además, el uso de la prueba de PCR puede permitir la rápida identificación del *C novyi* y los serotipos implicados.

Résumé - Infection par *Clostridium novyi* entraînant de la mortalité chez des truies élevées dans un système d'élevage extérieur en Espagne

Clostridium novyi était la cause suspectée de la mort de deux truies matures en gestation de race ibérique, sur la base d'un foie nécrotique rempli de gaz, d'une décomposition rapide et de tympanisme des carcasses, et de l'absence d'autres causes détectables de mortalité. Des cultures anaérobiques ont permis la croissance d'un grand nombre de micro-organismes apparentés à des clostridies, et *C novyi* type B a été identifié à l'aide d'une réaction d'amplification en chaîne par la polymérase (PCR) multiplex. Dans les cas de mortalité soudaine chez des truies en gestation, les vétérinaires doivent être au fait des causes les plus courantes de la mort, incluant l'infection par *C novyi*. Afin d'arriver à un diagnostic correct, il est essentiel d'effectuer un examen post-mortem et de prélever des échantillons aussitôt que possible après le décès. De plus, l'utilisation de méthodes PCR pourrait permettre une identification rapide de *C novyi* et des types impliqués.

Sow culling and mortality are among the most important determinants of financial well-being in pig-breeding units.¹ Financial losses associated with high sow mortality include the value of lost sows and pigs, the cost of early female replacement, and depletion of sow-herd quality as culling is less intentional.^{2,3}

The Iberian pig is a unique autochthonous breed, perfectly adapted to the Mediterranean natural ecosystem in the southwest of the Iberian Peninsula. Traditionally, they are reared outdoors in long production cycles (12 to 18 months), with the finishing pigs grown by making use of natural resources, mainly acorns from evergreen

oaks (*Quercus ilex* and *Quercus rotundifolia*) and pasture.⁴ At least 1 hectare (2.47 acres) of healthy oak-wooded meadow is needed to raise a single pig (extensive production system).

Nowadays, the Iberian pig breed is popular because its meat and meat products have very little in common with those obtained from selected pigs raised under intensive conditions. The high acceptance of these products in the Spanish market has allowed the flourishing of a niche market of increasing importance and very high profits.

Case description

Two mature gestating sows died unexpectedly in mid-January in an Iberian pig-breeding unit during their confinement

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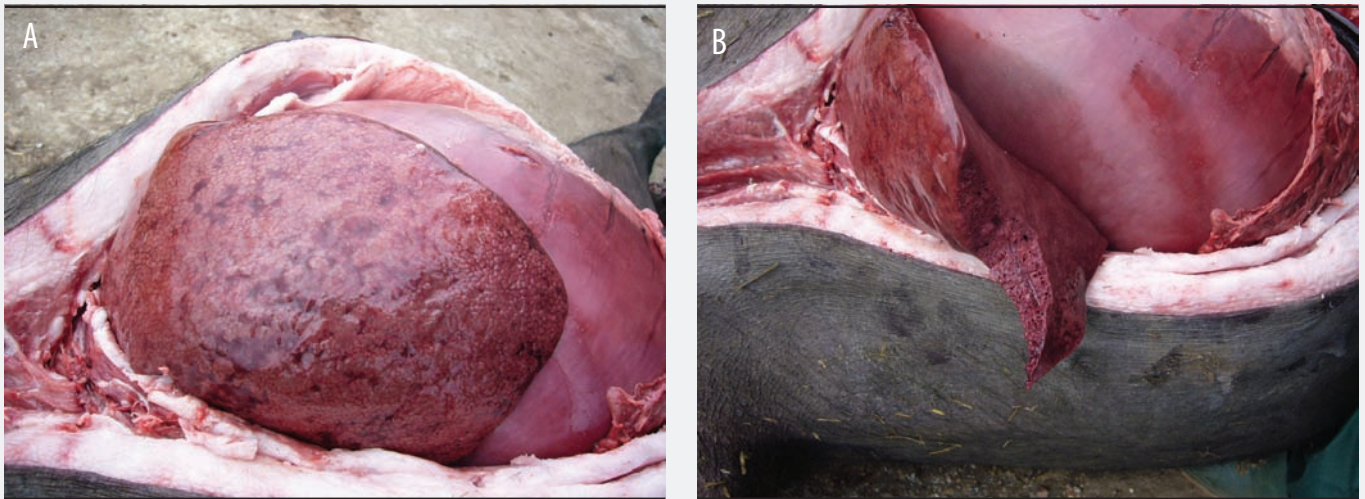
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Figure 1: The abdominal cavity of a mature gestating Iberian breed sow at necropsy. A: Full stomach and gas bubbles in the liver; B: The liver uniformly infiltrated with gas bubbles, presenting a spongy appearance on the cut surface, probably the most distinguishing feature of sudden death in sows caused by *Clostridium novyi*.



in farrowing crates. At present, on many Iberian pig farms, traditional outdoor sow gestation is followed by indoor farrowing in modern premises (semi-extensive management). These buildings are equipped with slatted flooring and natural ventilation. The case farm had 160 sows fed a commercial feed and with access to the natural meadowland resources of pasture and acorns. Sows farrowed twice a year and pregnant sows were confined in farrowing crates 1 week before giving birth.

Although postmortem examinations were performed only 2 or 3 hours after the death of the sows and ambient temperature was approximately 7°C, the carcasses were grossly distended and there was purple discoloration of the skin. Necropsy findings revealed generalized subcutaneous edema, a foul odor when the carcass was opened, enlarged and congested lymph nodes, bloodstained fluid in pleural, pericardial, and peritoneal cavities, serosal hemorrhages, and enlarged spleen. In each case, the stomach was full, the lungs were congested, and the liver was enlarged, friable, and dark, with gas bubbles uniformly infiltrated, thereby presenting a spongy appearance on the cut surface (Figure 1).

Necropsy samples were submitted to the School of Veterinary Sciences at the University of Extremadura (Cáceres, Spain) for examination of smears, culture, histopathology examination, and fecal flotation and sedimentation assays.

Histopathological examination of the liver of one sow revealed intrahepatic spherical non-staining cavities (gas bubbles) (Figure 2) and moderate multifocal lymphohistocytic hepatitis with hepatocellular degeneration and necrosis.

Large numbers of gram-positive rods were observed in Gram-stained smears from

the heart, lungs, kidneys, spleen, and liver of each sow (Figure 3). Anaerobic cultures yielded large number of *Clostridium*-like organisms. To make a rapid identification of pathogenic clostridia, the multiplex polymerase chain reaction (PCR) procedure described by Sasaki et al⁵ was performed. A BLAST homology

Figure 2: Histopathology section of the liver of an Iberian sow stained with hematoxylin and eosin showing hepatocellular degeneration and intrahepatic spherical non-staining cavities (gas bubbles) associated with *Clostridium novyi* infection (magnification ×100).

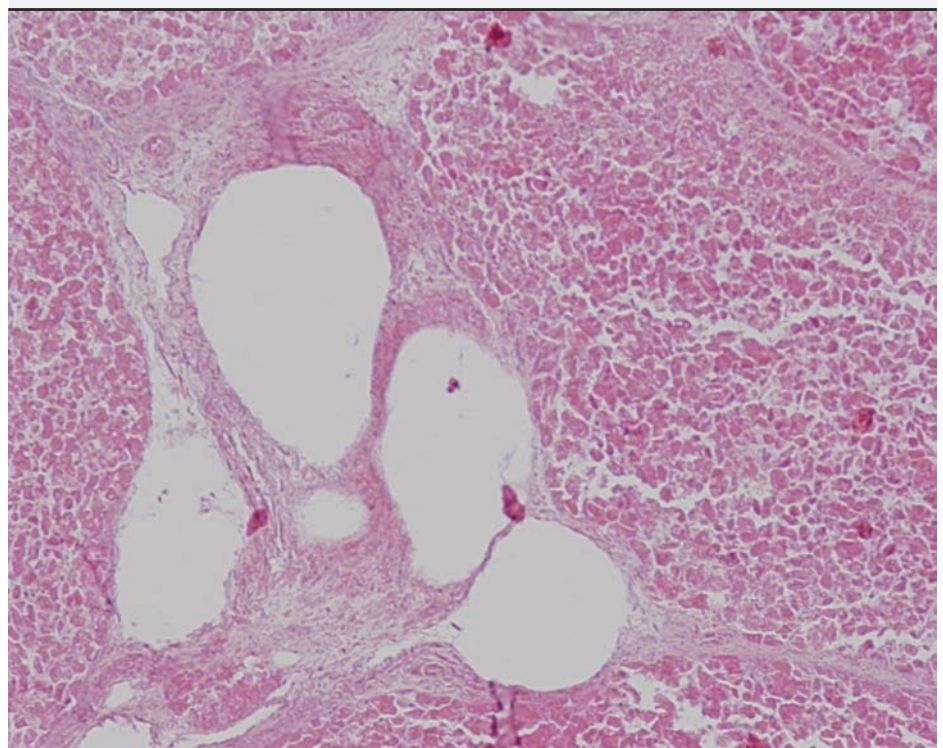


Figure 3: Smears from the liver of an Iberian sow that died of *Clostridium novyi* infection, showing large gram-positive rods with oval to cylindrical subterminal spores (magnification $\times 1000$).

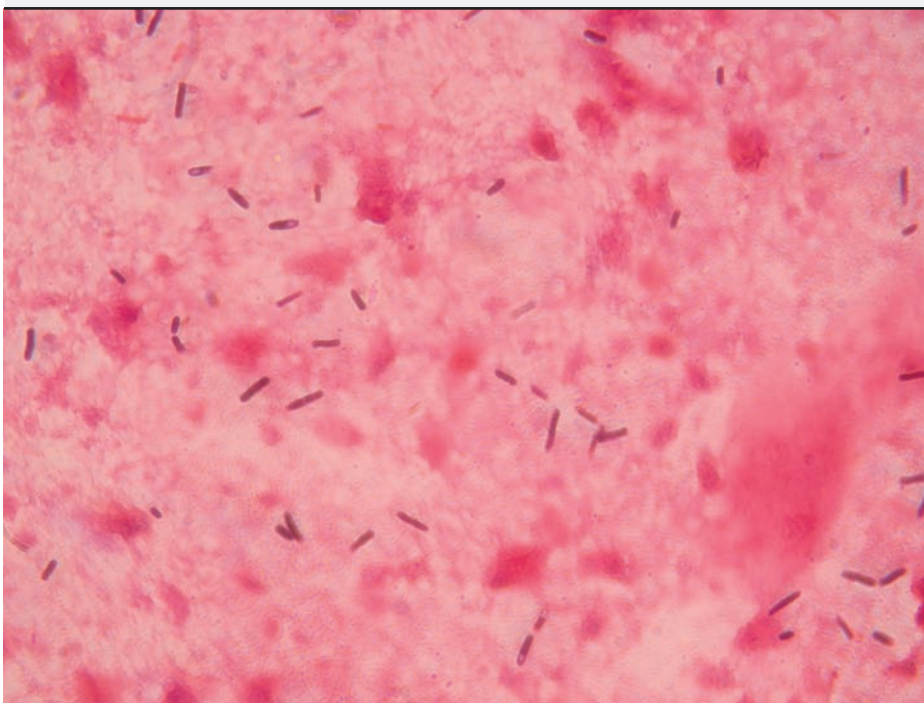
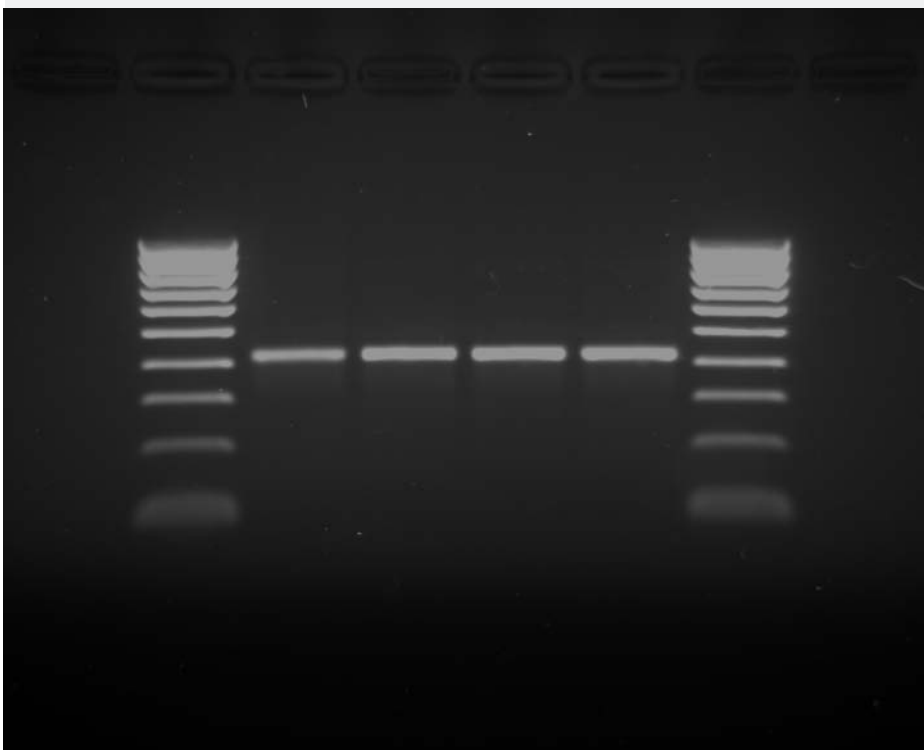


Figure 4: Gel electrophoresis (2% agarose gel stained with ethidium bromide) showing a species-specific 427-pb band identifying *Clostridium novyi* type B. The band was amplified using the multiplex polymerase chain reaction system described by Sasaki et al.⁵ Lanes 1 and 6: molecular weight marker ladder 1 kb (Bioline GmbH, Luckenwalde, Germany); lanes 2 to 5, PCR amplification products from duplicate positive cultures from the livers of two Iberian sows that died of *C novyi* infection.



search (program available at www.ncbi.nlm.nih.gov/BLAST) revealed that the 427-bp nucleotide sequence of the amplified product (Figure 4) matched the partial flagellin (*fliC*) gene of *Clostridium novyi* type B ATCC 25758 (DDBJ accession no. AB058936) (Figure 5).

Discussion

Clostridium novyi is an anaerobic, spore-forming, gram-positive rod that varies in size.⁶ The organism produces highly potent exotoxins (A to D)⁷ of which the lethal, necrotizing alpha toxin is considered to be the principal toxin of the type B strain in pigs.⁸ This toxin causes necrosis, increases permeability of the cell barrier, and disrupts intercellular junctions.^{6,9,10}

Clostridium novyi is the causative agent responsible for gas gangrene in humans and infectious necrotic hepatitis (black disease) in sheep, cattle, goats, and horses.⁷ Both *C novyi* types A and B have been isolated from reported cases of sudden death in sows.^{7,8}

Although *C novyi* infections are unusual in pigs,⁸ cases of sudden death in sows have been reported in intensive swine-breeding units in Europe^{7,11} and in outdoor pig units in eastern Europe.^{12,13} Nevertheless, to our knowledge, mortality of sows caused by *C novyi* has not been reported in Iberian pigs reared under extensive or semi-extensive conditions, an important livestock subsector in the southwest of the Iberian Peninsula.

The pathogenesis of *C novyi* sudden death in sows has not been elucidated. *Clostridium novyi* is a normal inhabitant of the large intestine and liver in pigs, although the route by which the organism reaches the liver has not been documented. If disease develops, spores in the liver become vegetative and produce potent exotoxins, responsible for the severe necrotizing and edematous tissue damage.⁷ In sheep with infectious necrotic hepatitis, previous damage to the liver parenchyma, usually by migrating liver flukes, is required for proliferation of *C novyi*.¹⁴ In this case, we did not find lesions of parasitic or larval migration in the liver, and the fecal flotation for intestinal nematodes was negative for each sow. However, an outbreak of swine dysentery had occurred in the herd. Some studies have reported that other concomitant low-grade infectious processes (eg, metritis, cystitis, enteritis) may predispose sows

Disease caused by *C novyi* can be controlled by reducing the incidence of pneumonia, metritis, and enteritis in affected groups of pigs. Several studies have reported the use of zinc bacitracin to reduce mortality, and disposal of carcasses by incineration or deep burial may reduce the contamination of the environment by spores.^{6,17,18} Prevention may also be achievable by the use of bacterin-toxoids or toxoids, and second-generation vaccines may be based upon native or recombinant alpha¹⁹ or beta toxoids. In contrast, vaccination of sows at risk with a multivalent clostridial vaccine does not seem to be an effective control measure.^{11,17}

Implications

- *Clostridium novyi* infection is a common cause of death in gestating sows.
- If carcasses are not examined soon after death and depending on weather and other postmortem conditions, it may not be possible to reach a diagnosis of *C novyi* infection.
- A timely postmortem examination and sample collection, along with microbial isolation and the use of PCR procedures, may allow a correct diagnosis of *C novyi* infection in swine.

Acknowledgments

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