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USE OF QPCR TO EVALUATE THE PRESENCE OF TYPE B *CLOSTRIDIUM NOVYI* IN SUDDEN DEATH SOWS AND HEALTHY SOWS.

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INTRODUCTION

Type B *Clostridium novyi* causes porcine infectious necrotic hepatitis, inducing sudden death (SD) in breeding sows. Little is known about the epidemiology of the disease, and its diagnosis is controversial since *C. novyi* is considered to be a common and early postmortem invader(1). Because isolation of this bacterium is not always successful due to its strict anaerobic requirements, and autolysis is inherent to the necrotic liver, the identification of *C. novyi* by PCR is a valuable tool to provide an aetiologic diagnosis. The aim of this study was to adapt a Type B *C. novyi* real time PCR (qPCR), as a tool to detect *C. novyi* in sows with and without suspected infection.

MATERIALS AND METHODS

A previously described PCR(2) assay was adapted to the SYBR® Green-based qPCR methodology. Validation was performed by testing the following specimens:

- **A) Bacterial strains:** A panel of 40 reference strains, including 31 non-type B *C. novyi* and *C. haemolyticum*, as well as 9 type B *C. novyi*. A set of 10-fold serial dilutions of the Type B *C. novyi* ATCC25758 strain was also tested.
- **B) Samples from sudden death sows:** Fifty liver samples from breeder sows on 25 unrelated commercial farms in Spain, France and Belgium. They were collected either fresh (29) or desiccated on FTA cards (21), from sows with suspected *C. novyi* infection based on gross lesions after SD.
- **C) Samples from healthy sows:** Individual rectal swabs from 270 healthy breeder sows housed on nine unrelated commercial farms in Spain, and 88 fresh liver samples from healthy breeding sows collected at slaughter (diverse origins).

Samples were subjected to automatic DNA extraction/purification (QIAamp DNA minikit in the Qiacube 2.0. Qiagen) and qPCR amplification (SYBR® Green qPCR Kit. Qiagen). The threshold cycle (Ct) value was determined for each sample.

RESULTS

The qPCR was 100% specific to type B *C. novyi*, with a limit of detection of 103 CFU/mL and efficiency of 95.6%.

Table 1. Results of the qPCR assay on samples of diverse nature and condition.

Origin	Sample type	Positive/ Total (%)
Bacterial culture	Type B *C. novyi* single CFU	9/9 (100)
Bacterial culture	Non-type B *C. novyi* single CFU	0/31 (0)
Sudden death sows	Fresh liver	19/29 (65.5)
	Desiccated liver (FTA card)	19/21(90.5)
Healthy sows	Fresh liver	0/88 (0)
	Rectal swab	0/270 (0)

CFU = Colony forming unit

CONCLUSIONS AND DISCUSSION

The qPCR assay was successfully adapted and validated, demonstrating suitability for the specific detection of Type B *C. novyi* DNA in both fresh and desiccated specimens. Additionally, results from this study demonstrate the usefulness of the FTA cards for room temperature transportation of complex biological samples such as necrotic liver in field cases of *C. novyi*-associated SD.

None of the samples, supposedly targets for *C. novyi*, such as the liver and rectal swabs from healthy sows, were found to be infected by this qPCR. These results differ from those reported in previously published studies dealing with this pathogen in pigs. Specifically, the assumption that *C. novyi* is a normal inhabitant of the posterior gut and liver of healthy pigs(3), is not supported in this study, suggesting that type B C. novyi infection is not ubiquitous.

All in all, this study demonstrates that qPCR would considerably improve the diagnosis of *C. novyi*-associated SD in sows, and provides relevant information for the establishment of preventive measures such as vaccination.

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