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PROCEEDINGS

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Comparison of conventional and real time PCR to detect *Mycoplasma suis* in Brazilian herds with reproductive disorders

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Introduction

Swine eperitrozoonosis (SE) has been affected herds in many countries around the world (2, 7). Clinical signs include icteroanemia, fever and decrease of reproductive performance mainly in the puerperal period leading to premature births, birth of weak piglets, low milk production and inadequate maternal behavior (6, 7). The laboratory diagnosis has been a frequent problem because *Mycoplasma suis* is not cultivable in vitro and there is no standard diagnostic tool. Diagnostics options comprehend the microorganism demonstration by blood smears, serologic and molecular tests (2, 5, 7). The goal of this study was to determine the *M. suis* incidence in herds with reproductive disorders using conventional PCR (cPCR) in comparison with Real Time PCR (qPCR).

Materials and Methods

Altogether, 188 animals were tested (sows n=80 and mummified or stillbirth fetuses n=88) from 27 herds in 2008. All fetuses were from these 80 sows of herds with reproductive disorders. *M. suis* DNA was extracted from blood of females and liver and spleen of fetuses with a commercial extraction kit. The cPCR reaction was optimized, using primers for 16S rRNA gene region, targeting a 839bp amplicon (5). The reaction mixture was heated at 94°C for 10 min, amplified for 32 cycles at 94°C for 1 min, 53°C for 1 min, 72°C for 2 min and then kept at 72°C for 10 min. The qPCR was performed using primers for a 16S rRNA region, targeting a 157bp amplicon (3). The reaction was optimized with 2µL of a *M. suis* DNA plasmid (pMsuis), 12.5µL of TaqMan Universal PCR Master Mix (Applied Biosystems), 20µM of forward and reverse primers and 10µM of probe. Detections were performed with an ABI Prism 7500 sequence detection system under the following conditions: uracil-N-glycosylase was activated at 50°C for 2 min, followed by PCR activation at 95°C for 10 min and 40 cycles of amplification (15 sec at 95°C and 1 min at 60°C). Serial 10-fold dilutions of *M suis* were prepared in 10 mM Tris-EDTA buffer.

Results

All fetuses tissues were cPCR and qPCR negative. All sows blood samples were cPCR negative and 14 out of 80 (17.5%) from 11 different herds were qPCR positive. The standard curve of qPCR was linear ranging from $3.28x10^9$ to $3.28x10^0$ copies/µL with a 0.99 square of correlation coefficient (R^2 value) and cycle threshold (*Ct*) values ranging from 8.33 to 37.60. Sensitivity analysis of qPCR showed a detection limit of $3.28x10^2$ copies/µL, while cPCR detected up to $3.28x10^7$ copies/µL. The specificity assay was negative with related bacteria (*M. hyopneumoniae*, *M. hyorhinis* and *M. flocullare*), only showed fluorescence with *M. suis*. The reproducibility assay of qPCR had an intra-assay variation from 1.25 to 4.62%, while an inter-assay variation ranged from 1.43 to 5.58%.

Discussion

Several countries described the SE *suis* occurrence but the real prevalence is not determined yet probably due to absence of a standard diagnostic method (2, 7). The tropism of the *M suis* by porcine erythrocytes (1) and the fetuses autolysis could explain the negative result in fetus from a positive dam. Besides that, other reproductive disorders could also lead to stillbirths and mummified and should be differentiated (6, 7). As expected, the qPCR was more sensitivity than cPCR and can be used as a valuable tool to detect M suis DNA (3, 5). The occurrence, epidemiology and genetic diversity of *M suis* are currently being investigated in order to achieve a better understanding of the *M suis* significance within the swine population.

References

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