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A novel double recognition enzyme-linked immunosorbent assay based on the nucleocapsid protein for early detection of European porcine reproductive and respiratory syndrome virus infection

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ABSTRACT

Precise and rapid detection of porcine reproductive respiratory syndrome virus (PRRSV) infection in swine farms is critical. Improvement of control procedures, such as testing incoming gilt and surveillance of seronegative herds requires more rapid and sensitive methods. However, standard serological techniques detect mainly IgG antibodies. A double recognition enzyme-linked immunosorbent assay (DR-ELISA) was developed for detection of antibodies specific to European PRRSV. This new assay can recognize both IgM and IgG antibodies to PRSSV which might be useful for detecting in routine surveillance assays pigs that are in the very early stages of infection and missed by conventional assays detecting only IgG antibodies. DR-ELISA is based on the double recognition of antigen by antibody. In this study, the recombinant nucleocapsid protein (N) of PRRSV was used both as the coating and the enzyme-conjugated antigen. To evaluate the sensitivity of the assay at early stages of the infection, sera from 69 pigs infected with PRRSV were collected during successive days post infection (pi) and tested. While standard methods showed low sensitivity rates before day 14 pi, DR-ELISA detected 88.4% seropositive samples at day 7 showing greater sensitivity at early stages of the infection. Further studies were carried out to assess the efficiency of the new assay, and the results showed DR-ELISA to be a sensitive and accurate method for early diagnosis of EU-PRRSV infection.

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1. Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is an enveloped, single-stranded positive-sense RNA virus that belongs to the family *Arteriviridae*, order *Nidovirales* (Cavanagh, 1997). Viral isolates have been classified in two different genotypes: type 1 PPRSV (European-PPRSV) and type 2 (US-PRRSV) (Nelson et al., 1993). PRRSV has been reported as a significant cause of swine morbidity and mortality worldwide (Albina, 1997). Consequently, the swine industry has been affected dramatically by PPRSV, suffering major economic losses every year (Neumann et al., 2005).

The viral genome is approximately 15 kb in length and contains nine known open reading frames (ORFs); ORF7 encodes the conserved nucleocapsid protein (N) (Mounir et al., 1995). The N protein is a small, basic, multi-functional protein with a molecular weight of 15 kDa, and has been identified as the most abundant and immunogenic viral protein (Nelson et al., 1994; Wootton et al., 1998). Such features make the N protein a suitable antigen for analysis of the immune response to PRRSV infection.

After PRRSV infection, pigs develop both antibody and cell-mediated immune responses. The IgM antibodies are detected first at day 5 post infection (pi) reaching a maximum peak at day 7 pi, and then declining rapidly to low levels by day 21 pi. The IgGs are detected first 7–10 days pi. After this period, IgG levels remain constant for months and decline finally to low levels by 300 days pi (Batista et al., 2004; Joo et al., 1997; Mulupuri et al., 2008).

Standard ELISAs are used widely for routine and experimental serodiagnosis of PRRSV (Brown et al., 2009; Chu et al., 2009; Diaz et al., 2005; Sorensen et al., 1997; Takikawa et al., 1996). However, these assays detect mainly IgG antibodies and have low sensitivity for IgMs, thus detection of infected pigs is not possible until day 7 pi. Prompt detection of PRRSV in swine farms is critical in order to carry out effective PRRS control, which could decrease the economic loss associated with an outbreak.

The aim of this study was to develop and optimize a rapid and sensitive assay to recognize not only IgGs but also other immunoglobulins, such as IgMs, allowing for early detection of EU-PRRSV infection. To accomplish this aim, PRRSV recombinant N protein was expressed in *Escherichia coli* and used as the coated and enzyme-conjugated antigen in a double recognition enzyme-linked

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