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Improving ante mortem diagnosis of *Erysipelothrix rhusiopathiae* infection by use of oral fluids for bacterial, nucleic acid, and antibody detection

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ABSTRACT

Swine erysipelas is an economically important disease caused by *Erysipelothrix rhusiopathiae*. Pen-based collection of oral fluids has recently been utilized for monitoring infection dynamics in swine operations. The diagnostic performance of bacterial isolation, real-time PCR, and antibody detection by enzyme-linked immunosorbent assay (ELISA) and fluorescent microbead-based immunoassay (FMIA) methods were evaluated on pen-based oral fluid samples from pigs experimentally infected with *E. rhusiopathiae* ($n=112$) and from negative controls ($n=32$). While real-time PCR was a sensitive method with an overall detection rate of 100% (7/7 pens) one day post inoculation (dpi), *E. rhusiopathiae* was successfully isolated in only 28.6% (2/7 pens). Anti-*Erysipelothrix* IgM and IgG antibodies in pen-based oral fluids were detected at 4 to 5 dpi by FMIA and at 5 and 8 dpi by ELISA. The number of infected animals per pen, and in particular the timing of antimicrobial treatment administration impacted bacterial isolation and ELISA results. In oral fluid field samples, *E. rhusiopathiae* DNA was found in 23.3% of the samples while anti-*E. rhusiopathiae* IgG and IgM antibodies were found in 59.6% and 5.5% of the samples, respectively. The results suggest that an algorithm integrating oral fluids as specimen and real-time PCR and FMIA as detection methods is effective for earlier detection of an erysipelas outbreak thereby allowing for a more effective treatment outcome.

1. Introduction

Swine erysipelas caused by *Erysipelothrix rhusiopathiae* continues to remain an important disease of pigs often associated with mortality and poor growth performance. It is estimated that 30–50% of domestic pigs worldwide carry the bacterium in tonsils or lymph nodes thereby contributing to continuous circulation of the disease (Opriessnig and Wood, 2012). Acute swine erysipelas is characterized by a sudden onset of the disease with fever, lethargy, and lameness. Acutely infected pigs are sometimes just found dead; however, many progress to develop characteristic diamond-shaped skin lesions. Sub-acute swine erysipelas often remains unnoticed while chronic *E. rhusiopathiae* infection may cause lameness due to chronic arthritis in affected pig populations.

Controlling *E. rhusiopathiae* can be hindered by a lack of reliable ante mortem tools for the real-time detection of bacterial circulation in pig populations. Acute *E. rhusiopathiae* infection is typically diagnosed postmortem and the current gold standard is isolation of the bacteria from suspect lesions (Bender et al., 2009). However, because of its small colony size, overall slow growth and frequent specimen contamination, *E. rhusiopathiae* often can be difficult to isolate (Wood and Harrington, 1978).

There are currently 28 known serotypes, with 1a, 1b, and 2 being the most frequent serotypes in pig populations (Takahashi et al., 1996; Imada et al., 2004; Opriessnig et al., 2004; Bender et al., 2011). In addition, there are several recognized genotypes including *E. rhusiopathiae*, *Erysipelothrix tonsillarum*, *Erysipelothrix inopinata* sp. nov., *Erysipelothrix* species strain 1, and *Erysipelothrix* species strain 2 (Norrung and Molin, 1991; Takahashi et al., 1992; Chooromoney et al., 1994; Verbarg et al., 2004; Takahashi et al., 2008). More recently, a novel multiplex real-time PCR assay, able to differentiate between *E. rhusiopathiae*, *E. tonsillarum* and *E.* species strain 2, has been described (Pal et al., 2009; Shen et al., 2010).

The collection and testing of oral fluids from pigs as a diagnostic and surveillance tool has been recently described (Prickett and Zimmerman, 2010) and has become widely adopted by swine veterinary practitioners. Oral fluid specimens are typically collected by hanging a rope in a pen and thereby collecting oral fluid from the majority of the pigs housed in that pen. Previous studies have validated the use of ante mortem collection of oral fluids for detection of porcine reproductive and respiratory syndrome virus (PRRSV) (Kittawornrat et al., 2012), bovine virus diarrhea virus (Terpstra and Wensvoort, 1997), foot-and-mouth disease virus (Alexandersen et al., 2003), porcine circovirus type 2 (Prickett et al., 2011), pseudorabies virus (Bouma et al., 1996), and vesicular stomatitis virus (Stallknecht et al., 1999). Extensive research on saliva samples, particularly in the last 20 years, has produced both PCR and antibody assays for a variety of human pathogens (HIV, hepatitis viruses, Epstein-Barr, measles, etc.) (Brandtzaeg, 2007).

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The impact of this research for swine production has been substantial. In contrast to serum samples, oral fluid collection in a pig population is user-friendly as one person is typically sufficient for the sample collection, non-invasive and not associated with stress to the pigs. It is also rather inexpensive, which has facilitated large surveillance studies and increased monitoring of certain pathogens in swine populations.

Pigs can be infected with *E. rhusiopathiae* infection by ingestion of contaminated feed or water or through skin abrasions (Opriessnig and Wood, 2012). Once infected, animals shed the organism in feces, urine, saliva and nasal secretions and successful isolation of *E. rhusiopathiae* or demonstration of its DNA in oral fluid samples has been described (Bender et al., 2010). Two novel serology assays, an *in-house* enzyme-linked immunosorbent assay (ELISA) (Giménez-Lirola et al., 2012a) and a fluorescent microbead-based immunoassay (FMIA) (Giménez-Lirola et al., 2012b) using a portion of the surface protective antigen (Spa) A, designated as SpaA415, were previously developed for detecting anti-*Erysipelothrix* spp. IgG antibodies in pig sera.

The objective of this work was to evaluate both direct (bacteria isolation and real-time PCR) and indirect (ELISA and FMIA) detection methods on pen-based oral fluid samples collected over time from pigs experimentally infected with *E. rhusiopathiae* and to establish a more effective diagnostic algorithm for monitoring *Erysipelothrix* spp. infection using oral fluids as the diagnostic specimen.

2. Materials and methods

2.1. Experimental samples of known infection status

2.1.1. Experiment design

To obtain oral fluids with known positive and negative *E. rhusiopathiae* status, pigs were challenged with *E. rhusiopathiae* serotypes 1a and 19. The details of the experimental design including housing and challenge have been described previously (Giménez-Lirola et al., 2012a). In brief, 33 approximately three-week-old mixed gender pigs were obtained from a herd confirmed free of *E. rhusiopathiae* and divided into different groups and rooms with 3 to 6 pigs in each (Fig. 1). One non-challenged group served as the negative control pen, six pens were challenged with *E. rhusiopathiae* reference strain E1-6P (serotype 1a), and one pen was challenged with *E. rhusiopathiae* reference strain 2019 (serotype 19). The serotype 1a reference strain E1-6P contains the *spaA* gene expressing the SpaA protein and the serotype 19 reference strain 2019 contains the *spaB* gene and expresses the SpaB protein (Shen et al., 2010). Four pigs co-housed with *E. rhusiopathiae* serotype 1a challenged pigs (groups 2A and 2B) served as contact controls to study the dilution effect (when infected and non-infected animals share the same space and thus both contribute to the pen saliva sample). To determine the effect of treatment on the diagnostic assays, the pigs in pens 2A, 2B, 3A, and 3B that developed clinical disease between day post inoculation (dpi) 2 and dpi 4 received a single dose of ceftiofur crystalline free acid (5 mg per kg; Excede®, Pfizer Animal Health, Inc.) intramuscularly. Similarly, pigs in pens 4A and 4B were treated with the same antibiotic at the same dose but prior to onset of clinical signs. Oral fluid samples were collected at dpi −7, 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 21 and 28, and divided into *Erysipelothrix* spp. positive samples ($n=112$) and negative samples ($n=32$) (Fig. 1). The oral fluid samples were tested for *Erysipelothrix* spp. using direct (bacterial isolation and real-time PCR) and indirect (ELISA and FMIA) detection methods. Blood samples were collected at dpi −7, 0, 7, 14, 21, 28 (Giménez-Lirola et al., 2012a) and sera tested by real-time PCR and for presence of anti-*E. rhusiopathiae* IgM antibodies by ELISA and FMIA. Previously it was determined by ELISA and FMIA that all negative control pigs remained seronegative for the duration of the study, whereas challenged pigs developed serum anti-*E. rhusiopathiae* IgG antibodies (Giménez-Lirola et al., 2012a, 2012b). The experimental design was approved by the Iowa State University Institutional Animal Care and Use Committee.

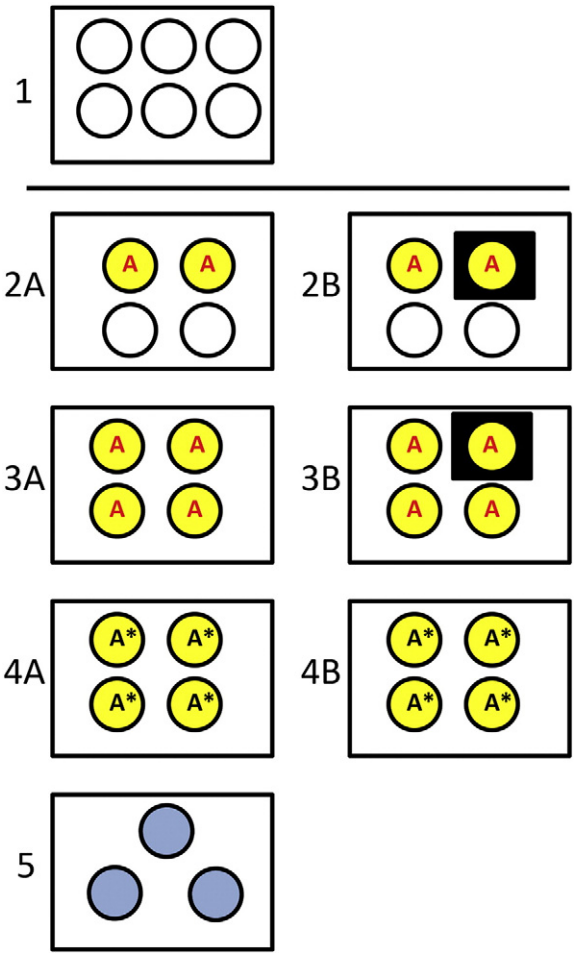

Fig. 1. Experimental design and numbers of samples collected. The number to the left of each pen corresponds to the pen ID and the circles inside the pens represent individual pigs. A white circle indicates that the pig was not infected, a yellow circle indicates that the pig was infected with *E. rhusiopathiae* 1a, a blue circle indicates that the pig was infected with *E. rhusiopathiae* serotype 19, a red capital A indicates that antimicrobial treatment was given after onset of clinical signs, and a black capital A with an asterisk (A*) indicates that antibiotic treatment was given before onset of clinical signs (2 days post inoculation or dpi). Circles within black squares indicate pigs that died from erysipelas at 2 dpi. Pen 1: Non-challenged negative control pen. Pens 2A and 2B: Half of the pigs remained non-challenged to determine the effect of bacterial load on detection. There were a total of 32 negative samples: 18 samples from pen 1 and 14 samples from pens 2 through 8 below (2 collections before inoculation). In addition, there were a total of 112 positive samples: 16 oral fluid samples collected from pens 2 thought 5 at days 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 21, and 28 after inoculation.

2.1.2. Sample collection

As a prerequisite for oral fluid collection, all animals were trained to chew the cotton rope prior to initiation of the study. Pen-based oral fluid samples were collected as described (Prickett et al., 2008; Kittawornrat et al., 2010, 2012). In brief, a section of cotton rope (Web Rigging Supply, Inc., Lake Barrington, IL, USA) was hung in each pen, using "rope holders" fixed at the front of each pen for 20–30 min allowing the pigs to chew on the rope. After the exposure period, the wet portion of the rope was inserted into a 1-gallon re-sealable plastic bag, the bottom portion of the rope was cut, and the bag sealed with the wet rope inside. At the laboratory, the oral fluid was mechanically extracted compressing the wet rope through a clothes wringer, causing the oral fluid to pool in the bottom of the bag. Thereafter, the bottom