RESEARCH REPORT
SWINE HEALTH

Title: Generating swine influenza virus (SIV) oral fluid diagnostic Reference Standards for community use - **NPB #12-052**

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INDUSTRY SUMMARY

Influenza A virus is an important component of the porcine respiratory disease complex (Thacker et al., 2001) and a pathogen with a major economic impact on swine production. Holtkamp (2007a,b) reported that influenza was among the three most costly swine infectious diseases in every stage of pig production (breeding, nursery, and finishing) in the U.S.

The public health issues related to SIV must also be taken seriously. The loss of export markets during the 2009-2010 H1N1 influenza pandemic cost U.S. producers $27 per pig (Anon, 2011). With 20% of the annual U.S. pork production exported to international markets (Anon, 2011), the economic viability of U.S. swine producers is synonymous with the public's perception of the health status of the U.S. national herd

This project was designed to accelerate the development of oral fluid-based SIV diagnostic assays by increasing the efficiency of researchers working in this area. Reference Standards make it possible to quickly and easily compare protocols and/or tests between laboratories because the results are based on the same, well-characterized specimens. This is an important gain in efficiency during assay development.

Once assays are implemented in the diagnostic laboratories, Reference Standards are used as "in house" controls. "In house" controls are run to monitor and verify assay performance (Statistical Process Control). Different diagnostic laboratories running the same "in house controls" are quickly able to compare and "trouble-shoot" the assays they run in common.

Thus, the purpose of this project was to develop SIV Reference Standards to be shared among laboratories, including researchers working on SIV diagnostic assay development and diagnosticians routinely running anti-SIV antibody assays.

KEYWORDS

Influenza A virus, diagnostics, oral fluid, antibody assays, PCR

SCIENTIFIC ABSTRACT

Influenza A virus is an important component of the porcine respiratory disease complex (Thacker et al., 2001) and a pathogen with a major economic impact on swine production. Holtkamp (2007a,b) reported that influenza was among the three most costly swine infectious diseases in every stage of pig production (breeding, nursery, and finishing) in the U.S.

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INTRODUCTION

In human diagnostic medicine, both PCR- and antibody-based assays for a variety of pathogens (HIV, hepatitis viruses, measles, etc.) have been developed for oral fluid specimens (Brandtzaeg 2007). In human medicine, the majority of oral fluid diagnostic assays are based on antibody detection. The presence of serum proteins in human oral fluid was first demonstrated in 1960 (Ellison et al., 1960). Shortly thereafter, Kraus and Konno (1963) reported that antibody was present in oral fluid only if it were also in the individual's serum. The non-invasive nature and low cost of oral fluid collection has facilitated extensive surveys of pathogens of public health importance, e.g., HIV in Africa and measles in Europe (Connolly et al., 2004; Ramsay et al., 1997).

As reviewed by Prickett and Zimmerman (2010), the presence of antibodies, pathogens, and acute phase proteins in oral fluids from swine and other animals reflects the findings in humans. Challacombe et al. (1978) proved that serum antibody passed from the circulatory system into the oral cavity by intravenously injecting radio-labeled antibody (IgG, IgM, IgA) into rhesus monkeys and subsequently detecting it in buccal fluids. Production of antibody in buccal tissues by serum-derived plasma cells in salivary glands and duct-associated lymphoid tissue (DALT) was also established during this period (Beckenkamp, 1985; Brandtzaeg, 1981, 1989; Crawford et al., 1975; Mestecky 1987, 1993; Morrier and

Barsotti, 1990; Nair and Schoeder, 1986). Plasma cells secreted IgA into saliva in conjunction with ductal and acinar epithelial cells expressing receptors specific for IgA. IgM and IgG were also found to be locally secreted, but at lower concentrations than IgA (Challacombe et al., 1995). Experimentally, tissues from the nasal and oral cavities of mice orally immunized with biodegradable microparticles were shown to contain IgA-secreting cells in salivary glands and nasal-associated lymphoid tissue (NALT) and the secreted antibody was demonstrated in oral fluid by ELISA at all sampling time points post-inoculation (Challacombe et al., 1997).

Corthier (1976) was the first to describe the presence of antibodies in swine oral fluids. Either intranasal or intramuscular inoculation with classical swine fever virus produced antibody responses measurable in serum and oral fluid (Corthier and Aynaud, 1977). Likewise, DeBuysscher and Dubois (1978) found that either oral or Thiry-Vella loop inoculation with E. coli caused the appearance of plasma cells in submandibular and sublingual salivary glands of pigs. DeBuysscher and Berman (1980) repeated this experiment with transmissible gastroenteritis virus (TGEV) and produced similar results. That is, either oral or Thiry-Vella loop inoculation produced an increase in the number of IgA- and IgM-secreting cells in salivary glands, with a small increase in the number of IgG-secreting cells. Loftager et al. (1993) found that IgA was detectable in oral fluid from pigs infected with Actinobacillus pleuropneumoniae, before it appeared in serum and concluded that oral fluid an antibody-based oral fluid assay could be a practical method to screen for APP infection.

Because of its economic importance, most oral fluid test development in pigs has focused on PCR-based (Chittick et al., 2011) and antibody-based (Kittawornrat et al., 2012; Langenhorst et al., 2011) assays for the detection PRRSV infection. However, most major swine pathogens and/or antibodies against them have also been reported in porcine saliva and/or oral fluids. A partial list would include: Actinobacillus pleuropneumoniae (Loftager et al., 1993), African swine fever virus (Greig and Plowright, 1970), classical swine fever virus (Corthier and Aynaud, 1977), Erysipelothrix rhusiopathiae (Bender et al., 2010), foot-and-mouth disease virus (Eblé et al., 2004), influenza A virus (Irwin et al., 2010), porcine circovirus type 2 (Prickett et al., 2011), PRRSV (Kittawornrat et al., 2010), torque teno virus (Ramirez et al., 2012), transmissible gastroenteritis virus (DeBuysscher and Berman, 1980), and vesicular stomatitis virus (Stallnecht et al., 1999). Cumulatively, the data support the view that oral fluid-based diagnostics have the potential for the detection of a broad range of infectious agents of swine.

OBJECTIVES

The broad objective of this project was to expedite the development of SIV oral fluid antibody assays. The specific aim of the proposed project was to develop oral fluid swine influenza virus (SIV) "Reference Standards".

MATERIALS AND METHODS

SIV-free pigs - 60 pigs free of anti-SIV maternal antibodies and free of SIV infection were transported to Iowa State University research facilities (November 30, 2012). Pigs were housed as 2 separate groups of 30 pigs each (one H1N1 group; one H3N2 group). All animals were confirmed free of SIV antibodies three times prior to SIV inoculation using an assay designed to detect any infection with influenza A viruses (MultiS-Screen® Antibody Test Kit, IDEXX Laboratories, Inc.). Thereafter, serum samples were collected weekly to monitor SIV antibody response and oral fluids were collected daily.

On December 29, 2012, one group of 30 pigs was inoculated with SIV isolate ISU-VDL1999035233 (H1N1). This field isolate represented H1 alpha and beta clades, i.e., this isolate displays unique antigenic

properties and appear to be broadly reactive to various clades for reasons that are unknown at present. The second group was inoculated with SIV isolate ISU-VDL2007013431 (H3N2). This field isolate represents cluster IV H3, i.e., it is a contemporary variant. Both viruses were isolated from diagnostic cases and are currently used as reference strains for hemagglutination-inhibition (HI) assays at ISU-VDL. The H1N1 virus has been shown to be cause clinical signs in pigs under experimental conditions. The H3N2 virus has not been used in any animal studies.

Once the project was completed, the SIV oral fluid Reference Standards will be available to researchers working on SIV diagnostic assay development and diagnosticians routinely running anti-SIV antibody assays.

RESULTS

Oral fluid and serum samples were successfully collected, as originally proposed. Testing confirmed that animals became infected with viral inoculae. Complete sample set were distributed to collaborators, as described in the project proposal.

DISCUSSION

Diagnostic assays based on oral fluid specimens have developed remarkably quickly, have been shown to be capable of excellent diagnostic performance, and have been accepted by producers and veterinarians. The approach we used in this proposal was unique in the sense that researchers from several institutions working on different facets of SIV oral fluids diagnostics collaborated so that they could work more efficiently to achieve the development of oral fluid-based SIV diagnostic assays.