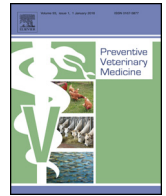




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Epidemiological factors associated to spread of porcine epidemic diarrhea in Japan

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ABSTRACT

Porcine epidemic diarrhea (PED) is an emerging disease of pigs that has recently led to large numbers of piglet deaths in a number of countries of Eastern Asia and The Americas. The objective of the present study was to identify and compare risk factors associated with PED infection in locally and non-locally PED-exposed farms in Japan. A questionnaire was administered to a convenience selection of pig farms located throughout Japan. Questionnaires were administered between November 2013 (when the first case was reported in Japan) and August 2014. PED-positive farms (cases, $n = 124$) were asked to provide information on their status (positive or negative) and select herd management practices for the two weeks prior to onset of PED clinical signs. Negative farms (controls, $n = 128$) were given the same questionnaire and asked herd management practices for the two weeks prior to a given reference date. This date was assigned based on the date of PED occurrence in the town/prefecture in which the farm was located. Case and control farms were categorized as “locally exposed” if they were located within a 5 km radius from a PED-infected farm and “non-locally exposed”, otherwise. Logistic regression analysis was used to identify factors associated with PED infection. Two separate regressions were done for locally exposed and non-locally exposed farms using PED status (positive/negative) as the dependent variable. PED in locally-exposed farms was associated ($P < 0.05$) with increased farm size (in 100 pig increments), shorter distances to the closest PED-positive farm (less than 1,001 m), and a disinfectant contact time of less than 20 min. In non-locally exposed farms, PED was associated ($P < 0.05$) with increased feed truck visits to the farm, no visit of the veterinarian, and again a disinfectant contact time of less than 20 min. These findings suggest that the mechanisms of PED spread in Japan were different for farms closer to case-farms compared to farms that were further away from PED cases. These results will contribute to understanding the epidemiology of the disease in Japan and will ultimately aid in designing and implementing effective prevention and control strategies in Japan and other regions epidemically infected by the PED virus.

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1. Introduction

Porcine epidemic diarrhea virus (PEDv) is an enveloped, single-stranded, positive-sense RNA virus of the genus *Alphacoronavirinae* in the family *Coronaviridae* that is related to the transmissible gastroenteritis (TGE) virus (Hofmann and Wyler, 1989; Saif et al., 2012). The PEDv emerged as a global threat to the swine industry in

2013, when a number of epidemics were reported in many important swine-producing countries of North America and Eastern Asia that were previously believed to be PEDv-free. These countries included US, Canada, and Mexico, including Korea, Taiwan, and Japan (Cima, 2013; More, 2013; Stevenson et al., 2013; Chen et al., 2014; Park et al., 2014; Hanke et al., 2015; Song et al., 2015). PEDv infection results in high morbidity and high mortality in <10 days old piglets, and decreased growth performance for those that survive infection (Huang et al., 2013; Mole, 2013; Stevenson et al., 2013; Alvarez et al., 2015b). PED was first reported in Japan in the 1990s, and a PED live vaccine was approved in 1996 (Sueyoshi et al., 1995). Since then, only isolated and relatively unimportant

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outbreaks have been recorded. However, in October 2013 the first PED outbreak was reported in Japan (Okinawa prefecture) after an interval of seven years. The virus rapidly spread across the country with 817 PED cases confirmed across 38 prefectures as of August 31, 2014 (Ministry of Agriculture, Forestry and Fisheries of Japan, 2014b). The PED strain detected in 2013 was genetically different from those circulating in the 1990s in Japan, and similar to the virus found in the US that same year (Nguyen et al., Submitted). Despite substantial human and financial resources being allocated to elucidate the mechanisms associated with the introduction and rapid spread of the virus (Ministry of Agriculture, Forestry and Fisheries of Japan, 2014b), there is still limited knowledge on the epidemiology of PEDv infection in Japan.

Variables previously identified as risk factors for PED spread in Japan include biosecurity practices, pig movement, and management during pig loading. These variables are also considered risk factors for PED spread worldwide (Zimmerman et al., 2012; Lowe et al., 2014). Feed has also been identified as a potential source for PEDv-spread in Japan following the infection with PED of a number of nucleus farms (Sasaki, personal communication). In these farms biosecurity protocols were particularly rigorous so feed was thought to be the only mechanism of invasion. The danger of feed transmission is consistent with studies performed in the US in which feed materials such as spray-dried porcine plasma were suspected to cause PED spread (Gerber et al., 2014; Pasick et al., 2014). Given the high infectiousness and low infectious dose of PEDv, proximity to an infected farm may also increase the risk of infection due to local spread (through aerosol transmission or contaminated fomites). This route is suggested by findings in Japan and the U.S. (Alvarez et al., 2015a; Sasaki et al., 2015).

Here, we hypothesized that factors associated with high risk of infection in Japan were different for farms in areas in which other infected farms were present (referred to as “locally exposed farms”) compared to farms that were further away from infected farms (referred to as “non-locally exposed farms”). Results will help to quantify the dynamics of PED spread and, ultimately, support the design and implementation of PED prevention and control measures in Japan and other epidemically-infected countries.

2. Materials and methods

2.1. Data collection

The study population included all pig farms that are consulted by clinical veterinarians affiliated with the Japan Association of Swine Practitioners (JASP). The JASP includes 52 veterinarians working in approximately 500 farms, which are approximately 10% of all herds in Japan. The country had 5270 herds in February 2014 (Ministry of Agriculture, Forestry and Fisheries of Japan, 2014a). Each veterinarian serves a range of 1–21 farms. In December 2014, all veterinarians in the JASP were invited to complete a questionnaire in relation to all PED positive farms (case farms) in their practice and an approximately equal number of control farms. Case farms were swine farms with a clinical presentation of PED and laboratory confirmation (RT-PCR). Control farms were farms from the same practice (selected by the veterinarian) with no history of PED infection. A questionnaire was administered to a convenience selection of pig farms located throughout Japan.

The questionnaire was designed to collect data regarding herd management practices. The questionnaire (Table 1) contained ten sections detailing herd management practices in the following areas: farm information, pig movement, use of own/borrowed equipment, feed, site visitors, manure application, biosecurity practices, pests and wildlife, biosecurity at pig loading/unloading, and disease status. The questionnaire (available upon request to the

first author) was pre-tested by a selected number of veterinarians regarding content, interpretation of questions, and responses.

Questionnaires were filled out between November 2013 (after the first case of the epidemic was detected in Japan) and August 2014. PED-positive farms (cases) were asked to provide information on their herd management practices for the two weeks prior to the initial detection of PED clinical signs in the farm. Negative farms (controls) were asked to provide the information for the two weeks prior to a reference date. This date was set for each control farm based on the first occurrence of PED in the town and prefecture in which the farm was located. Specifically, if PED had been detected in the same town, the reference date was defined as the date in which the first PED-positive farm was identified in that town. If PED had not been detected in the town of location of the control farm, the reference date was set as the date of the first outbreak in the closest town and, when no disease had been reported in the closest town, in the prefecture. If no PED had been reported in the same prefecture, the reference date was set as the date of detection of PED in the closest PED-positive prefecture. In Japan, all farms in which symptoms compatible with PED are detected must report this occurrence to the regional livestock hygiene center. If fecal samples from those farms showed RT-PCR positive (or PEDv RNA was detected by RT-PCR in the fecal samples), those were confirmed as a case farm. PEDv infection in all study farms was confirmed by RT-PCR and immunohistochemistry. We converted the farm name to an index number.

Case and control farms were categorized as “locally-exposed” (located within a 5 km radius from a PED-infected farm) and non-locally exposed, otherwise. The 5 km radius was selected because it was the spatial distance that maximized the observed-to-expected ratio of cases in Japan and the U.S., as reported elsewhere (Alvarez et al., 2015a; Sasaki et al., 2015).

2.2. Statistical analysis

All information was entered into a generic worksheet program and recoded into categorical data (nominal or ordinal level) for further analysis. Data were analyzed using the SAS software ver. 9.4 (SAS Institute Inc., Cary, NC, USA).

Two logistic regression analysis models were fitted to identify factors associated with PED: one in locally exposed and one in non-locally exposed farms. For both models, the response variable was PED-status (positive, negative). A univariate logistic regression analysis was performed to assess the association between PED status and each independent variable in the questionnaire (Table 1). Variables associated ($P < 0.25$) with PED were subsequently tested together in a multivariable logistic regression model. A backwards elimination procedure, that used a $P < 0.05$ selection threshold for the removal of non-significant variables, was used to identify main effects for the final model. Once main effects were obtained, two-way interactions were tested on the basis of biological plausibility. Although absence of independence associated with time and space may have been accounted for by the study design, location (four regional blocks in Japan) and time (month in which the farm provided information) were alternatively included as random effects in the multivariable model using the GLIMMIX procedure. The strength of association between variables and the response was quantified by computing the odds ratios (OR) and their corresponding 95% confidence intervals (CI).

3. Results

3.1. Descriptive statistics

Data were received from 42 veterinary practices, providing information for 258 (129 positive, 129 negative) farms. Some (2%,

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