

Case Report

An outbreak of classical swine fever in pigs in Bangladesh, 2015

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Abstract

In a group of 22 healthy pigs aged between 4 and 6 months, 2 pigs became ill with high fever, complete anorexia, cough and abnormal swaying movements on 22 June 2015. One of them died on June 24 and the second died on July 3. Shortly after, the remaining pigs also fell ill and died from the same illness by 10 August 2015. We investigated the aetiology, epidemiological and clinical features of the outbreak. We recorded the clinical signs and symptoms for each pig with the date of onset of illness. Veterinarians conducted *post-mortem* examinations on the 12 dead pigs, they collected tissue samples from the dead pigs and placed them in a tube containing 1 mL of nucleic acid extraction buffer (lysis buffer). We tested all the tissue samples by real-time reverse transcription polymerase chain reaction (rRT-PCR) to detect classical swine fever virus (CSFV) because the animals' symptoms matched those of this disease. We also conducted a phylogenetic analysis of the nucleotide sequence of the E2 gene segment of CSFV detected in a lung tissue sample. The attack rate (22/22) and the case fatality were 100%. The predominant symptoms of the disease included high fever, cough, diarrhoea and swaying movements of the hind legs prior to death. Of the 12 pigs tissue samples tested, all had evidence of the presence of CSFV RNA by rRT-PCR. The phylogenetic analysis indicated that the virus belongs to genotype 2.2, which is closely related to CSFV genotype 2.2 reported in India. Our investigation suggests that CSF is circulating in pigs, posing a risk for communities in Bangladesh that rely on pigs for economic income and dietary protein. Future research could focus on estimating the disease and economic burden of CSFV in pig rearing areas to determine if interventions might be warranted or cost-effective.

Keywords: Bangladesh, classical swine fever, outbreak, pig.

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Introduction

Classical swine fever virus (CSFV), a member of the *Pestivirus* genus within the Flaviviridae family (Meyers *et al.* 1989), is a highly contagious and often fatal infection in pigs which contributes to economic loss in pig farming (Edwards *et al.* 2000; Moennig *et al.* 2003; Postel *et al.* 2012) by constraining pig production and diminishing international trade in regions with CSFV-infected pigs and pig products (Boender *et al.* 2008; Sarma *et al.* 2008). CSFV has three major genotypes: 1, 2 and 3, and each genotype is categorized into three to four subtypes (Paton *et al.* 2000).

CSF has been reported in most Indian states (first being reported in 1962; Patil *et al.* 2010), and in

Nepal (Jha *et al.* 2012) and Bhutan (Monger 2015), which have also reported sporadic CSF outbreaks in their countries.

CSF has been controlled and eradicated from most of the countries using vaccination campaigns (Van Oirschot 2003; Greiser-Wilke & Moennig 2004; Song *et al.* 2013; Luo *et al.* 2014). However, CSF remains a threat to pigs raised in South Asia, including India, Nepal and Bhutan (Gatenby & Chemjong 1992; Dukpa *et al.* 2011; Prasad *et al.* 2011).

India has CSF prevention and control measures in place including available locally produced CSF vaccines (Bett *et al.* 2012), Bhutan has CSF vaccination programs for government breeding farms only (Monger 2015) and Nepal has planned to implement the

national classical swine fever control program (Animal Health, Kathmandu, Nepal). In Bangladesh, there is an absence of official CSF control measures, including CSF vaccination (Department of Livestock Service, Bangladesh).

CSF is a notifiable disease in India, Nepal and Bhutan (Monger 2015) and Bangladesh (Department of Livestock Services, Bangladesh).

Pig production in Bangladesh is a highly decentralized production system, coupled with stigmatization (Nahar *et al.* 2012b), combined to make surveillance of swine diseases difficult. Although Bangladesh is a predominantly Muslim country, there are minority ethnic communities that raise non-descriptive indigenous types of pigs (Nahar *et al.* 2012b). In 2009, the Department of Livestock Services (DLS) estimated that there were ~200 000 pigs in the country (DLS, unpublished data). A study found that 88% of pigs were raised in a backyard farm (Khan *et al.* 2014). Most of the pig raisers were impoverished and invested minimally in pig feed, housing and preventive care (Nahar *et al.* 2012a,b). However, pig rearing is an important source of livelihood and animal protein for these communities in Bangladesh (Nahar *et al.* 2015).

Case report setting

In 2015, on day zero, we purchased 22 seemingly healthy pigs aged between 4 and 6 months from the northwestern Noagaon district to study the immunogenicity of the live attenuated Japanese encephalitis (JE) vaccine. On day 2, we moved the pigs to Gazipur, located in central Bangladesh (Fig. 1). We chose Gazipur because this is an urban area where JE is unlikely to circulate. Trained veterinarians administered one dose of the JE vaccine on day 5 by intramuscular injection. On the same day, prior to administering the vaccine, all pigs were bled to look for pre-existing antibodies to JE.

Case report

All pigs were kept in one confinement pen under continuous monitoring (observed 4 times a day) by a trained research assistant. Once the pigs showed illness (fever, cough or diarrhoea), the research assistant informed the study veterinarian about this illness, the study veterinarian then performed a clinical examination and the sick pigs

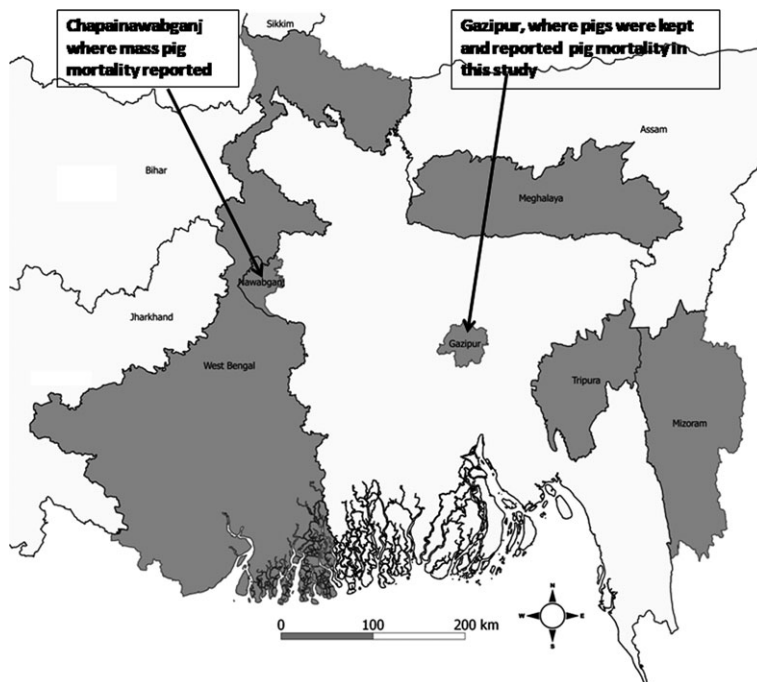


Fig. 1. Map showing the area where pigs were kept, where mass pig mortality occurred and various states (sharing borders with Bangladesh) of India which have reported classical swine fever (CSF) outbreak (shaded in grey).

were transferred to an isolation pen and provided with symptomatic and supportive treatment to those pigs.

Two pigs became ill with high fever, complete anorexia, cough and abnormal swaying movements the day after their vaccinations (on June 22). One died on June 24 and the second died on July 5. The pigs continued to fall ill and die due to the illness despite veterinarians treating the animals for their signs. Following the death of the first pig and with the continuing illness in the other pigs (e.g. high fever, cough, diarrhoea, complete anorexia, reluctance to move, shivering and swaying movements in the hind quarter for 2–10 days prior to their death), we suspected that the pigs might have contracted CSF infections. We investigated to confirm the causative agent and describe the epidemiological and clinical features of the outbreak.

Post-mortem examination

Trained veterinarians recorded the clinical signs of each pig with dates of onset of illness. To determine the clinical–pathological features, the team also conducted *post-mortem* examinations on 12 dead pigs (>50%); they collected tissue samples from the liver, kidneys, lungs, spleen, tonsils and brain of the pigs and placed each tissue sample in a tube containing 1 mL of nucleic acid extraction buffer (lysis buffer).

Laboratory investigations

We tested all the tissue samples by real-time one-step reverse transcription polymerase chain reaction (rRT-PCR) to detect CSFV (Dias *et al.* 2014).

All the pigs belonged to a single herd and were grouped together in one pen, and so were most likely infected with the same pathogen. Therefore, one randomly selected sample was subjected to sequence based analysis for CSFV genotyping. We prepared cDNA and subsequently performed PCR to amplify a 1342-bp segment of the E2 gene of CSFV. We sequenced the PCR product using Sanger's sequencing method (Jiang *et al.* 2013). We constructed a phylogenetic tree which included

the E2 sequence of our porcine strain (accession no.: KX345847) along with the related global strains deposited in the GenBank database by maximum likelihood reconstruction of sequence alignments using Molecular Evolutionary Genetics Analysis (MEGA) 6. We also calculated frequency, percentage and mean for the variables related to pig demographic characteristics and clinical manifestation.

Results

The mean age of the pigs was 4.5 months and their mean weight was 11.2 kg. The majority (55%) of the pigs were male. The infection rate (22/22) and the case fatality rate were 100%. The mean duration between illness onset and death was 4 days (range 0–13 days) (Fig. 2). Predominant signs included high fever (82%), cough (50%), diarrhoea (50%), huddling (41%) and swaying movements of the hind quarter (36%) prior to their death (Table 1). All pigs were dead by day 65 (Fig. 3). The gross changes recorded included congested lungs, a haemorrhagic and dark tan-coloured liver, focal haemorrhage, a black/tan-coloured spleen, a slightly haemorrhagic brain, enlarged tonsils and pinpoint haemorrhaging in the kidneys (Fig. 4). All 12 pig tissues samples tested positive for CSFV RNA by rRT-PCR.

We were able to amplify the E2 (1343 bp region) gene segment of a CSF positive lung tissue sample. The phylogenetic analysis indicates that it belongs to genotype 2.2 CSF virus, which is closely related to CSFV genotype 2.2 in India (95% at nucleotide level) (Fig. 5).

Discussion

The clinical, necropsy and molecular findings suggested that the pigs had the CSFV infection. We identified a high fatality rate in CSFV-infected pigs which is comparable to other studies (Laevens *et al.* 1999; Dewulf *et al.* 2000). In this outbreak, all pigs showed clinical signs comparable to other studies of CSFV (Moennig *et al.* 2003; Malmarugan *et al.* 2014). Moreover, the necropsy findings of this study are similar to the findings from other pigs that died

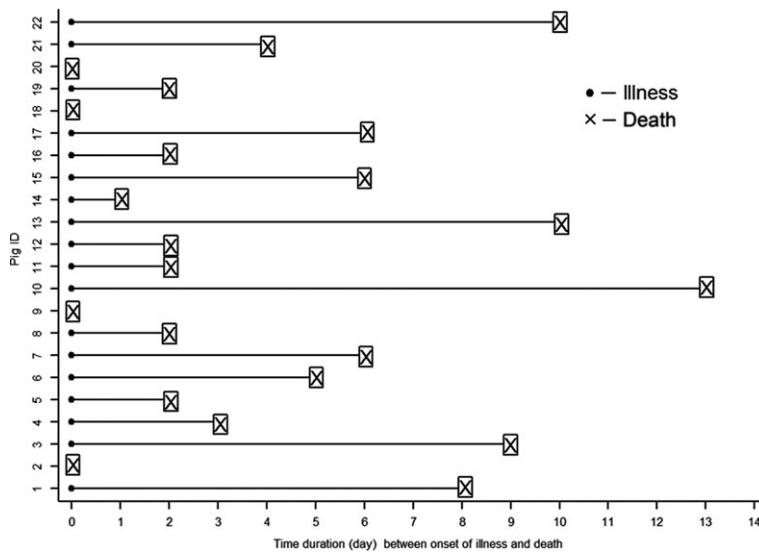


Fig. 2. Time duration between onset of illness and death of pigs ($n = 22$).

of CSFV infection in India (Ravishankar *et al.* 2007; Malmarugan *et al.* 2014). To the best of our knowledge, this is the first report of CSF-associated pig mortality in Bangladesh. However, CSF-associated pig mortality has already been reported in India, Nepal and Bhutan (Jha *et al.* 2012; Monger 2015; Khatoon *et al.* 2017).

We did not rule out the exact source of introduction of CSF in this study. However, we assume that the outbreak of CSF is likely to be due to introduction from the pig herd where we purchased these pigs. The pigs herd could be infected with CSF asymptomatically during purchasing of these pigs. We followed up with the pig herd owner over the phone about the signs and mortalities in their pig herd. They reported a similar illness (predominant signs were fever, cough, diarrhoea and reluctance to move prior to death) had occurred in their herds, indicating that these pig herds may have been exposed to CSFV. Moreover, a mass mortality of pigs raised in backyards in northwestern Bangladesh (Fig. 1) which is close to the border of India, with similar signs, was also reported in the year leading up to this event (Hasan Ali, personal communication). In addition, our CSFV sequence data revealed that the Bangladeshi strain of CSFV belongs to genotype 2.2. The strain exhibited highest identity (95% at nucleotide level) and a tight phylogenetic

affiliation (Fig. 5) with the Indian strains of CSFV genotype 2.2 found during 2008–2015 (Desai *et al.* 2010; Patil *et al.* 2010; Sarma *et al.* 2011). These findings also suggest that the source of introduction of CSF in this study could be the pig herd where we purchased our study pigs.

The onset of illness in two pigs was the day following vaccination (Fig. 3). The incubation period for CSFV is 2–14 days (Blome *et al.* 2017), which also suggests that these pigs were infected before they received the vaccine. In addition, we gave this same vaccine to 971 of pigs in the area on 27 June 2015,

Table 1. Demographic and clinical characteristics of CSFV-infected pigs in Bangladesh, 2015 ($n = 22$)

Variables	
Age (months), mean (SD)	4.5 (0.47)
Body weight (kg), mean (SD)	11.2 (1.97)
Sex, n (%)	
Male	12 (55)
Clinical manifestation, n (%)	
Fever	18 (82)
Cough	11 (50)
Diarrhoea	11 (50)
Complete anorexia	9 (41)
Huddling	9 (41)
Reluctance to move	9 (41)
Shivering	8 (36)
Swaying movement	8 (36)

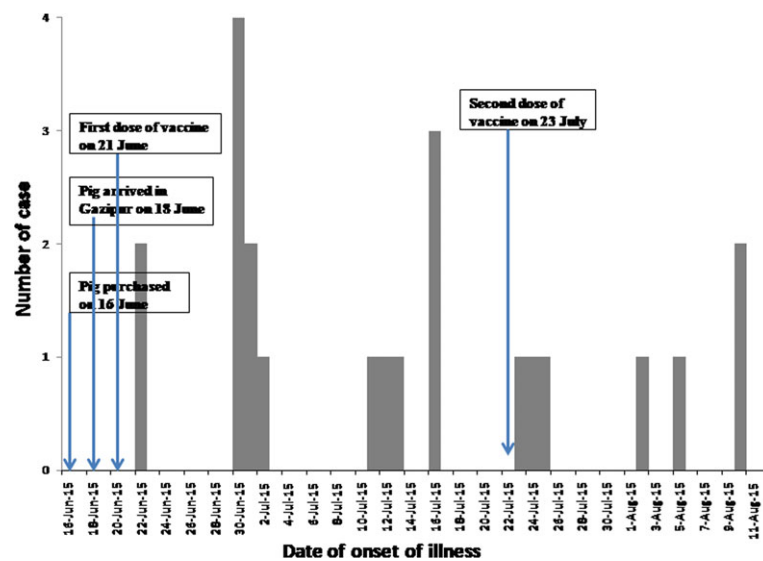


Fig. 3. Temporal distribution of date of onset of illness of classical swine fever (CSF) among pigs during this outbreak in Bangladesh, June–August 2015.

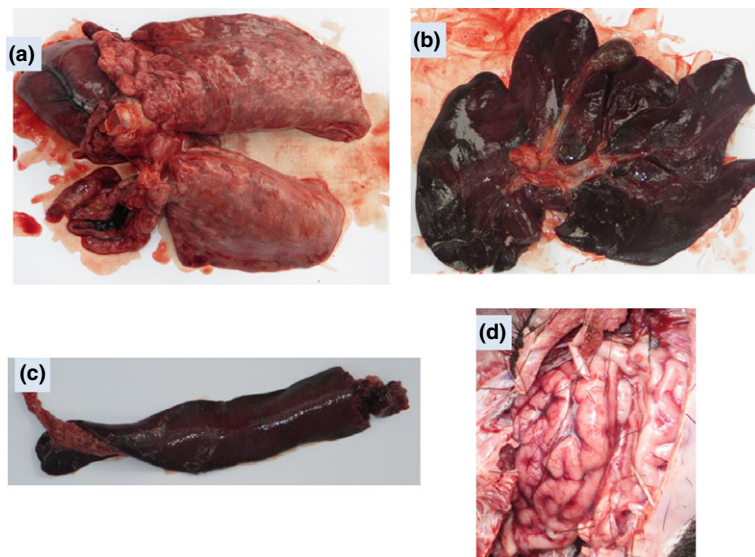


Fig. 4. Classical swine fever (CSF)-related lesions: (a) congested lungs, (b) haemorrhagic and dark tan-coloured liver, (c) haemorrhagic and dark tan-coloured spleen and (d) slightly haemorrhagic brain.

and did not receive any reports of illness in vaccinated pigs suggesting that the vaccine was not contaminated. These indicate that outbreaks were not caused by vaccination, but rather due to exposure prior to purchase.

Due to the lack of veterinary care, surveillance and diagnostic capacity in pig raising communities in Bangladesh (Nahar *et al.* 2012b), many outbreaks of CSF in pigs remain undiagnosed or underreported.

India uses locally produced CSFV vaccines as CSF prevention and control measures in northeastern

states of India (Bett *et al.* 2012). Bhutan uses locally produced CSFV vaccine in government pig breeding farms only (Monger 2015). Nepal has planned to implement the national CSF control program (Animal Health, Kathmandu, Nepal). Bangladesh is now exploring CSF surveillance and control options now that there are reports of its circulation.

Securing resources and support for prioritizing efforts to control CSF in Bangladesh will depend on compelling data about the disease, the economic burden of CSF and future research should include

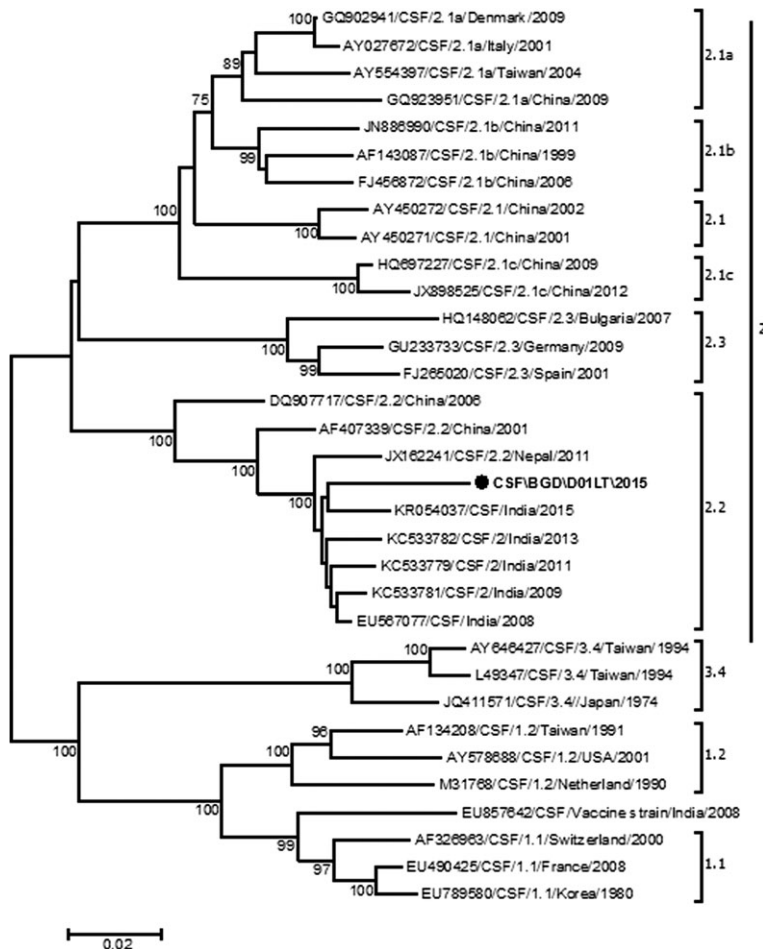


Fig. 5. Phylogenetic tree of partial nucleotide sequences (1343 bp) of classical swine fever virus (CSFV) E2 gene. Numbers indicate the bootstrap values (100 replicates), and only the values above 70% are shown in the figure. Horizontal distances are proportional to sequence distances. The circle indicates Bangladesh CSFV strain.

estimating this burden. Effective interventions have been implemented in India (Bett *et al.* 2012), which may be culturally and economically appropriate for Bangladesh if the disease burden justifies intervention.

Our study had limitations. We did not test the pre-vaccination serum samples of pigs for detecting CSF status (antibodies against CSFV). However, the finding of this study associated with unexpected event in accordance with our protocol. Moreover, the serum samples are no more available to do further analysis. Therefore, testing further for CSFV antibodies is beyond the scope of the study. In addition, all pigs were younger (mean age was 4.5 months) in this event, and we assume that low seroprevalence rate of CSFV in young pigs as it is supported by studies led by Kaden *et al.* (2000, 2002). Moreover, our virological

findings indicate that the pigs could be non-immune to CSFV; therefore, they were infected with CSFV as we detected CSFV RNA in their tissue samples.

Nucleotide sequence accession number

The nucleotide sequences of CSFV\BGD\DO1LT\2015 strain was deposited in GenBank under accession number KX345847.

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Conflict of interest

The authors declare that they have no conflicts of interest.

Contribution

Study design: SS, Sample testing: MEH, RH, MZR, Statistical analysis: SS, Manuscript draft: SS Revision and manuscript approval: SS, MEH, ESG, RH, MZR.

Ethics statement

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. The US National Research Council's guidelines for the Care and Use of Laboratory Animals were followed.

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