Detection of foot-and-mouth disease (FMD) virus in healthy cattle and buffalo at Southeast Asian slaughterhouses

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Abstract

Foot-and-mouth disease virus (FMDV) is widespread throughout much of the world, including parts of South East Asia. As part of the World Organisation for Animal Health (OIE)'s South East Asia and China Foot-and-Mouth Disease Project (SEACFMD), field sampling was performed to help understand evidence of widespread virus exposure observed previously. Serum and dry mucosal swabs were collected to evaluate the presence of FMDV RNA on the nasal, oral, and dorsal nasopharyngeal mucosal surfaces of 262 healthy cattle (n=38 in Laos; n=47 in Myanmar) and buffalo (n=12 in Laos; n=2 in Myanmar) immediately following slaughter in three slaughterhouses. Swabs and serum were tested by the OIE FMD world reference laboratory using pan-serotypic real-time reverse transcription-PCR (RT-PCR) and serum was evaluated using the FMD PrioCHECK non-structural protein (NSP) ELISA. In total, 7.3% of animals had detectable FMDV RNA in one or more of the three sites including 5.3% of nasopharyngeal swabs, 2.3% of oral swabs, and 1.5% of nasal swabs. In all animals, serum was found not to contain detectable FMDV RNA, and 37.8% of animals were positive for NSP antibodies, indicating likely past exposure to FMDV. Results were comparable for Laos and Myanmar, and were similar for both cattle and buffalo. The current study demonstrates the utility of detection by swabbing the nasopharynx in the post-mortem context, in situations such as post-mortem where probang samples are not feasible. Additionally, FMDV present on the oral and nasal mucosa of clinically-healthy large ruminants in Laos and Myanmar, if viable, may potentially play a role in the epidemiology of FMD in these countries, and perhaps more widely within Southeast Asia.

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Summary

Foot-and-mouth disease virus (FMDV) is widespread throughout much of the world, including parts of South East Asia. As part of the World Organisation for Animal Health (OIE)'s South East Asia and China Foot-and-Mouth Disease Project (SEACFMD), field sampling was performed to help understand evidence of widespread virus exposure observed previously. Serum and dry mucosal swabs were collected to evaluate the presence of FMDV RNA on the nasal, oral, and dorsal nasopharyngeal mucosal surfaces of 262 healthy cattle (n=38 in Laos; n=47 in Myanmar) and buffalo (n=12 in Laos; n=2 in Myanmar) immediately following slaughter in three slaughterhouses. Swabs and serum were tested by the OIE FMD world reference laboratory using pan-serotypic real-time reverse transcription-PCR (RT-PCR) and serum was evaluated using the FMD PrioCHECK non-structural protein (NSP) ELISA. In total, 7.3% of animals had detectable FMDV RNA in one or more of the three sites including 5.3% of nasopharyngeal swabs, 2.3% of oral swabs, and 1.5% of nasal swabs. In all animals, serum was found not to contain detectable FMDV RNA, and 37.8% of animals were positive for NSP antibodies, indicating likely past exposure to FMDV. Results were comparable for Laos and Myanmar, and were similar for both cattle and buffalo. The current study demonstrates the utility of detection by swabbing the nasopharynx in the post-mortem context, in situations such as post-mortem where probang samples are not feasible. Additionally, FMDV present on the oral and nasal mucosa of clinically-healthy large ruminants in Laos and Myanmar, if viable, may potentially play a role in the epidemiology of FMD in these countries, and perhaps more widely within Southeast Asia.

Keywords Abattoirs, Buffaloes, Cattle, Foot-and-Mouth Disease, Foot-and-Mouth Disease Virus, Reverse Transcriptase Polymerase Chain Reaction

Introduction

Foot-and-mouth disease virus (FMDV) is a contagious virus of cloven-hoofed ungulates (Artiodactyla), of major trade-limiting importance (Anonymous, 2020b). FMD is present in approximately two-thirds of the world's countries, in which it acts as a barrier to trade. Parameters of acute FMD infection in naive cattle are well-defined, and continue to be refined by research (Arzt et al., 2010; Stenfeldt et al., 2015). In contrast, the subclinical cycles of FMD in the approximately 128 countries where the virus circulates are less-well documented. Experimental work to assess viral shedding and immune response in recently vaccinated, previously naive animals (Parthiban et al., 2015) has partially filled gaps in knowledge of the virus in animals with humoral immunity. In endemic regions, FMD lesions similar to that reported in epidemics are documented, but the characteristic, "fulminant" herd-wide disease may be observed or reported inconsistently

compared to what is documented in epidemic contexts (van Andel et al., 2020b; Bertram et al., 2018). The driving factors in the discrepancy between viral circulation and frequency of clinical FMD in endemic regions have not been well-described. Almost fifty years ago, Anderson et al. (1974) warned of Kenyan cattle where "the occurrence of clinical outbreaks does not necessarily give a true assessment of the amount of virus in the environment as subclinical or inapparent infection could occur, particularly in partially immune cattle." Much uncertainty remains about how FMD manifests in endemically-infected herds, and the various states by which subclinical infection exists considering the distinct phases: neoteric and persistent infection (Stenfeldt and Arzt, 2020).

Before considering endemic disease, it is useful to briefly review the well-defined stages of epidemic disease in naive cattle (Yadav et al., 2019). The majority of naive cattle exposed to FMDV will become infected through exposure of the mucosa of the upper respiratory tract. In an individual animal, there is a 2-14 day incubation period; a pre-clinical viraemia typically within 48-72 hours of infection. Viraemia occurs 24-48 hours after initial detection of virus within nasopharyngeal mucosa (Pacheco et al., 2016). Virus can also be secreted through breath, milk, faeces, saliva, urine, and semen up to 4 days prior to clinical illness. A 2-3 day period of clinical illness follows, and within 24 hours there is rupture of epithelial vesicles, which slough and erode, creating the classical fulminant lesions of FMD (Anonymous, 2020b). Resolution occurs in a characteristic pattern by local reepithelialisation of erosions by remaining islands of basal epithelial cells. Full disease resolution occurs by day 15 from the start of clinical infection. Antibodies are a useful indicator of natural disease, and may be partially cross-protective to future infection from other serovars (Garland, 1974). Cattle with viral infection past 28 days are defined as chronic carriers, and in these cattle, virus most commonly persists in the dorsal nasopharyngeal mucosa (Stenfeldt et al. 2016; Stenfeldt and Arzt 2020). In live chronic carriers, the probang technique provides the most useful sample of the area of viral persistence, allowing for molecular detection and virus isolation (Barend et al., 2016; Stenfeldt and Arzt 2020), however scraping this area with a cuvette at slaughter also yields virus (Anderson et al., 1974). Readers are directed to the recent review by Stenfeldt and Arzt (2020) for a more detailed summary of the chronic carrier state.

Besides the chronic carrier state, other factors that influence subclinical infection in cattle include: the pre-clinical (pro-dromal or neoteric) state; previous infection and natural immunity to a particular serotype; vaccination status; and potential breed or species resistance associated with host-adaptation. Protective immunity is not mentioned as a possible factor in subclinical infection in any document we could find, except when antibodies were a product of vaccination (Kitching, 2002, Sutmoller P., Casas Olascoaga R. 2002). This omission is interesting, since seroprevalence is one of the major differences between FMDV-free cattle and cattle in endemically-infected populations. In endemically-infected populations, the seroprevalence of the population as a whole, by definition, is expected to be greater than zero. In fact, reports suggest that seroprevalence in some cases is much higher than zero: in non-vaccinated populations of large ruminants, region-based seroprevalence is reported at between 19-71% in Africa (Eldaghayes et al. 2017; Munsey et al. 2019; Wungak et al 2016), and between 18-51% in Asia (de Carvalho Ferreira, 2017; Dukpa et al., 2011; Blacksell et al. 2008).

Knowledge of in-country FMD epidemiology forms one of the major requirements for countries participating in the Food and Agriculture Organisation of the United Nations (FAO) and the World Organisation for Animal Health (OIE)'s Progressive Control Programme for FMD (PCP-FMD), which provides a benchmarking guide to countries wishing to progress towards FMD freedom (Jamal & Belsham, 2013). Multiple factors, including the naturally-acquired immunity of endemic herds, may potentially influence the patterns of FMD observed in endemic regions. The majority of research on FMD has been either experimental or has occurred during outbreaks in previously-FMD-free regions. Reports of differences in disease presentation and epidemiology in endemic regions present striking contrasts to those from epidemic contexts, but the reports themselves are limited in number. For example, while 90% of FMDV-infected naive cattle would be expected to develop disease upon exposure, FMDV-exposed cattle in an unvaccinated herd in Cameroon had a seroprevalence increase of 30% over a twelve month observation period, despite the fact that only 6 of 100 animals were noted to have FMD-associated lesions during this time (Bertram et al, 2018). In addition, clinically-healthy cattle and buffalo from Pakistan produced milk containing live FMDV up to 6 months

following FMD outbreaks on the same farms (Nawaz, 2019; Ahmed 2017). These examples suggest that cattle and buffalo exposed early and frequently to FMDV might exist in a different relationship with the virus than is commonly understood in an epidemic context.

Control of FMD has been identified as a priority for the livestock production sectors in Myanmar and Lao PDR, due to their perceived role in regional transmission of FMD due to cattle movement (Blacksell et al., 2019). Previous studies undertaken in these countries as part of the PCP-FMD demonstrated widespread exposure of cattle and the circulation of multiple strains of FMDV (Bo et al, 2019; Blacksell et al., 2008; Khounsy et al. 2008; van Andel, 2020b) similar to that reported in other endemic regions. Concerningly, analyses of outbreak reporting in Myanmar failed to demonstrate that seroprevalence could be accurately predicted either by government-level outbreak reports (van Andel, 2020a), or by villager or headman observations (van Andel, 2020b), suggesting that more research is needed to better understand the FMD epidemiology in Myanmar.

As part of understanding the circulation of FMDV in Southeast Asia, we examined the subclinical presence of FMDV on the dorsal nasopharyngeal, oral, and nasal mucosal surface of healthy Laos and Myanmar cattle and buffalo at slaughter. Research was undertaken as part of the South East Asia and China Foot-and-Mouth Disease Project (SEACFMD) supported by OIE and the New Zealand Ministry for Foreign Affairs and Trade (MFAT) and the New Zealand Ministry for Primary Industries (MPI). We obtained post-mortem dry swabs and sera from 232 clinically-healthy adult large ruminants, which were tested by real-time PCR and non-structural protein (NSP) ELISA, respectively. The results of this study may help better inform the epidemiology of FMDV in the Southeast Asian region.

Materials and Methods

Slaughterhouse (SH) sites: sampling occurred at Dongdou SH and Nongduang SH (Vientiane, Laos) during May and June, 2019; and at Mandalay City SH, Mandalay, Myanmar during July and August, 2019 (Figure 1). Animal contact at the slaughterhouses varied – at Dongdou animals were gathered in a chute up to 12 hours prior to slaughter, and secured to a railing with a headrope. At the other slaughterhouses, animals typically arrived in small groups by truck up to 24 hours prior to slaughter and were tethered by a headrope to trucks or within the slaughterhouse, or kept in stalls with animals from one trader together until the time of slaughter. Animals from one trader could represent multiple original villages, districts or townships of origin. Animals were brought by traders to the slaughterhouse in groups, collected from different villages and transported together. For the Myanmar slaughterhouse, animals from 16 traders were sampled over five nights. In Laos, all animals tested at Dongdou were supplied by one trader, but at Nongduang (a more traditional slaughterhouse), animals were supplied by 10 traders over three nights.

Study population: Healthy cattle and buffalo bound for human consumption were sampled opportunistically post-mortem. Complete samples were obtained from 132 animals (84 cattle and 48 buffalo) in Laos, and 130 animals (5 buffalo and 125 cattle) in Myanmar.

Samples: a sample set including serum and swabs from three sites (nasals, oral, and dorsal nasopharyngeal) were collected from each animal. Whole blood was collected in 10 mL red-top (plain) Vacutainer tubes (Becton, Dickinson and Company, Franklin Lakes, New Jersey, USA) following severing of the jugular vein as part of the normal slaughter process. Tubes were either centrifuged at 1500 x g for 3 minutes, or left to clot within 12 hours of collection). Serum (1 mL) was then collected into a 1.5 mL screw cap tube (Sarstedt) and frozen at -80degC until processing. Plain dry rayon Copan^{TM} swabs (Copan Diagnostics Inc., Murrieta, California USA) were collected from the sites mentioned above during the slaughter process, were immediately inserted into a cryovial containing 1.0 mL of DNA/RNA Shield^{TM} (Zymo Research), and were kept chilled on ice packs for between 3 to 12 hours until arrival at the local laboratory where they were frozen at -80degC for transport to the World Reference Laboratory for FMD at the Pirbright Institute (Ash Road, Surrey, UK).

Swabs were collected in the following manner: oral swabs were rubbed for approximately 3 s on the hard palate, buccal surfaces, and tongue as possible given the position of the animal, and were inserted up to

the length of the 15cm swab; nasal swabs were rubbed on all inside surfaces of both nostrils for 1-2 s each, with the swab inserted maximally into the nasal openings; pharyngeal samples prioritised sampling of the dorsal pharyngeal mucosa, the site of optimal experimental FMDV retrieval for persistently-infected animals (Pacheco et al., 2015) and involved insertion of the swab from a caudal direction through the oesophagus, with blind manual guidance to the dorsal nasopharyngeal mucosa, which was rubbed vigorously. During initial sampling, dissection of a buffalo head confirmed that palpation of landmarks allowed sampling of the target mucosal surface. Where ruminal contamination was present, heads were pre-washed with water from a hose or bucket to minimise contamination of swabs. During collection, field staff employed frequent changing of gloves to prevent cross-contamination. Environmental control samples were collected each 10-15 carcasses by waving swabs through air adjacent to carcass collection sites (air controls) and by immersing swabs in local hose or trough water (water controls).

Data collected at sampling: at the time of swabbing, oral and nasal lesions and any signs of lameness were assessed and recorded. Animal species, age and sex were collected from traders at the time of sampling. At the time of sampling, the external nares and rostral oral cavity were observed for the presence of gross lesions including vesicles, erosions or swellings, and evidence of excessive salivation.

Laboratory assays: The frozen serum and swab samples were maintained at -80degC at the National Animal Health Laboratory of the country of origin, then transported to Pirbright Institute on dry ice (Ash Road, Surrey, UK). Serum was evaluated for FMDV non-structural proteins (NSPs) using the FMD PrioCHECK NSP ELISA as per kit instructions with the exception that two wells were used per sample. Swabs were evaluated for the presence of FMDV RNA by the pan-serotypic one-step real-time reverse transcription-PCR (RT-PCR) (Callahan et al, 2002). RT-PCR values were determined to be positive if the cycle threshold (CT) value was under 40.

Analysis: analyses were performed in R (R version 4.0.0 (2020-04-24) Copyright (C) 2020 The R Foundation for Statistical Computing).

Results

Across all animals, 3.4% (9/262, 95% CI 1.58-6.42%) had detectable FMDV RNA in oral and/or nasal swabs. When all swab types (oral, nasal and pharyngeal) were included, 7.3% (19/262, 95% CI 4.42-11.09%) of all animals in both countries had detectable FMDV RNA on at least one swab (Table 1), including 10.4% (5/48, 95% CI 3.47-22.66%) buffalo and 6.0% (5/84, 95% CI 1.96-13.34%) cattle in Laos, and 0% (0/5, 95% CI 0.00-52.18%) buffalo and 7.2% (9/125, 95% CI 3.34-13.23%) cattle in Myanmar. No animals had any lesions or clinical signs consistent with FMD. The most common site of detection in animals with a positive real-time RT PCR CT value was dorsal nasopharyngeal (14/19 animals), followed by oral (6/19 animals) and then nasal (4/19 animals). Animals positive on at least one swab were significantly more seropositive (61.9% seropositive, 13/21) compared with animals from the whole group (101/262, 38.5%) (Chi-square statistic = 4.4084, p-value = 0.036). All environmental control swabs were negative by real time PCR.

Fifty animals in the study group were male (19%), and the rest were female except for one animal with unrecorded sex. Sex was not a significant factor in whether there were one or more positive swabs (1 positive male to 19 positive total) or 5.3%, Fisher's exact test 95% CI (0.01-1.46), p-value = 0.137. More young animals (under 24 months of age) were sampled in Laos (n =37) compared to Myanmar (n =4). The mean age of animals that were positive on one or more swab was 4.5 years, and age was not significantly different compared to animals that were negative on all swabs (Mann-Whitney U test, W = 2545.5, p-value = 0.4532). Animals with positive RT-PCR results were spread across multiple traders and across multiple nights.

Discussion

The concept of FMD as a fulminant, outbreak-driven animal emergency is appropriate for considering the disease within FMD-free countries. Useful as it is, the concept has subsequently been "imported" into countries where FMDV is widespread and endemic, and where virus presence is known to exist without ongoing clinical outbreaks (Farooq et al., 2018; de Carvalho Ferreira et al. 2017). Although outbreaks

occur frequently in many endemic regions, they are not sufficient to explain the high seroprevalence in many regions where no recent clinical outbreak has been reported (van Andel, 2020b).

We found that 2.3% of healthy, commercially-slaughtered large ruminants from an endemic region had detectable FMDV RNA within the oral cavity, and that 1.5% had FMD RNA in the nasal cavity. The results were similar for both Laos and Myanmar, suggesting this might be a pattern which is common throughout the region. This is the first study to sample the oral and nasal mucosal surface of a healthy population of large ruminants within an endemic region, and adds to a small but growing literature examining the state of FMDV infection in animals from endemic regions. By comparison, in naive cattle, oral and nasal shedding following experimental infection sharply declines over 21 days (Parthiban et al. 2015) with extinction of virus in the oral or nasal mucosa regardless of whether animals clear infection or become chronic carriers (Stenfeldt et al., 2016). By contrast, the presence of FMDV RNA within the dorsal nasopharynx, has been well-established by multiple other studies (Stenfeldt et al., 2016; Barend et al 2016; among others) which have defined the role of that region in the chronic carrier state. Although FMDV detection in the nasopharynx is typically demonstrated by collection of oropharyngeal fluid from live animals with a probang cup, the current study demonstrates the utility of detection by swabbing the nasopharynx in the post-mortem context.

The animals we sampled were chosen by convenience, and nothing was known about their previous exposure to FMDV except that they originated from an FMD-endemic region. None of the animals in this study had visible scars, lesions or other clinical signs (e.g. salivation, lameness) suggestive of current or recent FMD. We used NSP ELISA positivity as an imperfect proxy for prior exposure, as a way of judging whether any viral excretion might be related to pre-clinical acute FMD infection. Vaccination with whole vaccines (which could cause NSP-positive reactors) is thought to be low in both Laos and Myanmar, and was not considered likely to influence NSP ELISA results. Animals with detectable mucosal viral RNA at any site were significantly more likely to be seropositive than the sampled population, but not all RNA-positive animals had antibodies. Since development of antibodies postdates initial infection and early replication, we hypothesized that animals with detectable FMDV RNA but no antibodies might have been in the early (neoteric) stages of infection (Stenfeldt and Arzt, 2020). Six seropositive animals had detectable viral RNA in the oral and nasal cavity. Since NSP serology is not serotype specific, one possible reason for shedding is that these animals did not have protective antibodies to the same virus that was detected by RT-PCR.

One benefit of slaughterhouses is that they aggregate animals and present a single site for sampling many animals with minimal handling complications compared to field sampling. Others have used slaughterhouse sampling as a way of accessing FMDV samples, including in Pakistan and Kenya: Navid et al (2019) found that 11% of buffalo tested at Pakistani slaughterhouses carried detectable FMDV antigen in their probang sample. Anderson et al. (1974) found that over 3% of Kenyan cattle at slaughter had viable virus in pharyngeal scrapings. However, animals sent for slaughter might not be indicative of a population at large, and more work remains to be done before it is clear whether our results can be extrapolated to the general large ruminant population of Laos and Myanmar. In addition, animals transported for slaughter are more likely to have undergone stress and mixing, with potential new infection or reactivation of viral activity. The potential role of stress in FMDV reactivation has been an area of interest, although experiments using immunomodulatory drugs have been unable to effect active shedding of virus in chronic carriers (reviewed more thoroughly in Stenfeldt and Arzt, 2020).

Outbreaks of active FMD in Myanmar and Laos have been attributed to incursions of new strains of FMDV (Bo et al, 2019; Khounsey et al. 2008). Our results suggest the interesting possibility that in addition to these virus "incursions", the epidemiology of FMDV spread within endemic contexts could include subclinical viral infection and shedding within populations of apparently healthy animals. Dissecting the pathophysiology of infection in each animal in our study was not attempted, since too much was unknown about previous exposure and infection. Our intent was to evaluate a cross-section of healthy animals for a possible role in viral shedding, to inform a regional epidemiological understanding. Descriptions of viral location in chronic carrier cattle and buffalo focus heavily on nasopharyngeal virus detection (Stenfeldt et al., 2016; Moonen et al. 2004; de Carvalho Ferreira et al. 2017). Our results suggest that perhaps more attention

should be paid to other mucosal surfaces, and that the relationship between FMDV and large ruminants in endemic settings may differ from that predicted experimentally. If small numbers of cattle and buffalo contain viable virus on environmentally-accessible mucosal surfaces such as the nose and mouth, there is the potential for low-level transmission of virus on a regular basis between animals in close contact. This might help explain the phenomena seen in FMDV-enzootic contexts, where seroprevalence rises without disease (Bertram et al, 2018), and where outbreak reporting imperfectly predicts seroprevalence (van Andel 2020b). The observation that healthy dairy cattle and buffalo shed virus in their milk (Nawaz, 2019; Ahmed 2017), raises the possibility that in endemic countries, some cattle might be exposed from birth to both virus and protective maternal antibody. Additionally, intensive sampling of buffalo in a dairy colony in Pakistan demonstrated that within one year numerous serotypes and strains could move through a herd without any clinical signs of infection (DOI: 10.1111/tbed.12963).

A limitation of this study is that we cannot be sure the presence of viral RNA is indicative of the presence of viable FMDV. In addition, although environmental control swabs were negative, the possibility of cross contamination within different anatomical sites or between animals cannot be discounted. Positive results however were widespread throughout the sampling period and locations, suggesting contamination was not a prominent cause of positivity. Dry swabs were employed for sampling rather than metal cuvettes used at slaughter by Anderson et al. (1974); this was to prevent possible contamination between animals. Swabs were also considered an affordable, useful surveillance tool that might enable use of the technique by agencies seeking to increase their PCP-FMD surveillance options. Because we chose not to obtain probang samples prior to slaughter in order to prevent possible contamination of sites, our swab-based dorsal nasopharyngeal results are not comparable with results from other studies. Future studies should make use of virus isolation to determine presence of viable virus in animals with detectable FMDV RNA, and should work to establish the relative sensitivity of the post-mortem swab-based technique with more established techniques such as the probang.

In summary, we found that 5.3% of healthy, asymptomatic cattle and buffalo sampled during slaughter in Laos and Myanmar had detectable FMDV RNA in their dorsal nasopharynx, and 3.4% had detectable FMDV RNA in their oral and/or nasal cavities. Although the viability of this viral RNA is unclear, our results suggest the possibility that FMDV present on the oral and nasal mucosa of asymptomatic large ruminants could play a role in the epidemiology of FMD in Southeast Asia. It is known that surveillance approaches focussing solely on clinical outbreaks of FMD may not be sufficient to detect all circulating virus in populations of large ruminants. Yet, field sampling of live animals is highly labor-intensive and often logistically prohibitive. The current study demonstrates the utility of detection of FMDV RNA by swabbing of the nasopharyngeal, oral and nasal mucosa in a post-mortem context, and may constitute a useful complementary tool for surveillance in endemic regions.

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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. No ethical approval was required as samples were collected post-mortem.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of Interest Statement

The authors declare no conflict of interest.

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Tables

Table 1: PCR and NSP ELISA results from healthy cattle and buffalo (n=262) tested at slaughter in Laos (Dongdou and Nongduang) and Myanmar (Mandalay). Swab samples for RT-PCR were taken from three mucosal surfaces (oral = OR; nasal = NS; dorsal nasopharynx = DNP). Real-time PCR results were considered positive for detection of FMDV RNA at any CT value under 40.

		NSP ELISA Interp	**NS PCR pos**	**OR PCR pos**	**DNP PCR pos**	**PCR pos any s**
Laos	cattle	38/84 (45.2%)	4/84 (4.8%)	1/84 (1.2%)	3/84 (3.6%)	5/84 (6.0%)
	buffalo	12/48 (25.0%)	0/48 (0%)	2/48 (4.2%)	4/48 (8.3%)	5/48 (10.4%)
	total	50/132 (37.9%)	4/132 (3.0%)	3/132 (2.3%)	7/132 (5.3%)	10/132 (7.6%)
Myanmar	cattle	47/125 (37.6%)	0/125 (0%)	3/125 (2.4%)	7/125 (5.6%)	9/125 (7.2%)
	buffalo	2/5 (40.0%)	0/5 (0%)	0/5 (0%)	0/5 (0%)	0/5 (0%)
	total	49/130 (37.7%)	0/130 (0%)	3/130 (2.3%)	7/130 (5.4%)	9/130 (6.9%)
Cattle total		85/209 (40.7%)	4/209 (1.9%)	4/209 (1.9%)	10/209 (4.8%)	14/209 (6.7%)
Buffalo total		14/53 (26.4%)	0/53 (0%)	2/53 (3.8%)	4/53 (7.5%)	5/53 (9.4%)
Total		99/262 (37.8%)	4/262 (1.5%)	6/262 (2.3%)	14/262 (5.3%)	19/262 (7.3%)

Figure legends

Figure 1. Slaughterhouse locations in Mandalay, Myanmar and Vientiane, Laos. Mandalay slaughterhouse = diamond symbol, Dongdou slaughterhouse = star symbol, Nangduang slaughterhouse = balloon symbol.