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Serological and clinical surveillance studies to validate reported foot-and-mouth disease free status in Tsirang district of Bhutan

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ABSTRACT

Serological and clinical studies were conducted between March 2009 and August 2010 to validate the foot-and-mouth disease free status of Tsirang district of Bhutan as determined by the country's passive surveillance system. Randomised (first survey) and targeted (third survey) samplings, with subsequent follow-up samplings (second and fourth), were conducted on FMD-susceptible animals to detect the disease at a design prevalence of 25% and 20% at the individual animal-level and village-level, respectively. Sera from cattle, goats, pigs, and sheep were tested for the presence of non-structural protein (NSP) antibodies using two commercial (PrioCHECK® FMDV NS and CHEKIT®-FMD-3ABC-bo-ov) and one in-house NSP kit (c-ELISA, AAHL, Australia). The overall seropositivity (all species) at the animal-level was 3% (95% CI: 1.7, 4.8) and 3.5% (95% CI: 2.1, 5.4), for the randomised and targeted surveys, respectively. Except for one goat from the first survey, none of the small ruminants and pigs had NSP antibodies. The seropositives from the first and targeted surveys were distributed among 13 and 16 of 20 villages sampled, respectively. All repeat testing from the initial seropositive animals and their herd mates, for both the first and third surveys, were negative in the NSP tests 6–8 months later. Using the hypergeometric exact probability formula for two-stage analyses, the results enabled rejection of the null hypothesis and supported conclusion that the population was free from disease at the minimum expected prevalence of 20% at the 95.53% and 99.46% confidence levels, for the randomised and targeted surveys, respectively. Clinical surveillance also showed absence of disease or clinical signs suggestive of FMD. The few seropositives were likely to be false positives due to factors such as imperfect specificities of the tests and possible NSP-residues in the vaccines. The study has paved the way for initiation of zoning approaches for the progressive control of FMD in Bhutan.

1. Introduction

Foot-and-mouth disease (FMD) is a highly contagious viral disease that affects all cloven-hoofed domestic animals including cattle, sheep, goats, pigs and buffalo (OIE, 2008). The virus, belonging to the Aphthovirus genus of the family Picornaviridae, has seven clinically indistinguishable serotypes: O, A, Asia 1, SAT 1, SAT 2, SAT 3 and C. Foot-and-mouth disease is endemic in Bhutan and although serotypes O, A, Asia 1 and C have been recorded, serotype O is the most predominant (Dukpa et al., 2011a). The measures adopted to control FMD in Bhutan include vaccination (prophylactic and post-outbreak); regulation of movement of livestock and livestock products during outbreaks; application of zoo-sanitary measures; and an awareness programme for the farming community

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(Anonymous, 2005). Trivalent FMD vaccines (serotypes O, A, and Asia 1) manufactured in India are used for vaccination of mainly cattle. The frequency and coverage of vaccination varies as per the disease status of the districts. Bhutan continues to experience outbreaks of FMD almost every year despite an active control programme. Several factors have been cited as reasons for this, including inadequate vaccination coverage, ineffective vaccination due to difficulties in maintaining cold chain of vaccines, difficulties in enforcing livestock movement bans, mountainous topography, and lack of knowledge about the epidemiology of the disease (Dukpa et al., 2011a). As FMD is endemic in most districts in Bhutan, controlling the disease for the whole country would be expensive and unrealistic as has been shown over the years (Dukpa et al., 2011a). This is further compounded by the fact that the country shares a long and open border with the Indian states of Assam, West Bengal and Arunachal Pradesh where FMD is endemic (Sarma and Sutopa, 2003; Bhattacharya et al., 2005; Sanjoy and Sarma, 2005).

Given the budgetary constraints for disease control activities, and the continued risk of incursion of new strains of FMDV through unregulated movement of animals across international borders (Dukpa et al., 2011c), alternative approaches are needed to effectively control FMD in Bhutan. There is an increasing worldwide call for the use of progressive control of FMD using the concepts of zoning (Fujita, 2004; Rweyemamu et al., 2008). Zoning is an approach aimed at identifying geographical areas of varying disease status within a country or region for the purpose of trade, disease control and eradication (Anonymous, 2007). The OIE-led project, South-East Asia and China foot-and-mouth disease (SEACFMD) control and eradication campaign – uses this concept of progressive zoning (Edwards and Abila, 2004; Edwards, 2005) as one of the key elements to eradicate FMD from the region by 2020. This approach is used given the limited resources for the FMD control programme both from within the participating countries as well as from the donor countries (Abila, 2010) for the SEACFMD. The aim is to progressively upgrade areas from infected to control zones; from control to eradication zones, from eradication to free zones with vaccination; and ultimately to free zones without vaccination (Anonymous, 2007).

Although FMD is endemic in most districts in Bhutan (Dukpa et al., 2011a), according to the country's passive surveillance system, the disease has not been reported in Tsirang district since 1998 (Anonymous, 2009b). During the outbreak in Tsirang in 1998, only cattle were affected and no serotyping was done. This district shares a border with three other districts (Dagana, Wangdue Phodrang, Sarpang) where the disease is endemic (Anonymous, 2009b). Owing to natural barriers consisting of rivers, huge cliffs and dense forests, there is a good potential for this district to maintain its reported disease free status. However, epidemiological studies need to be conducted to confirm the reported disease free status before the initiation of a zoning approach (Rweyemamu et al., 2008; Oo, 2010). This will then pave the way for application of a progressive zoning approach in Bhutan as an alternative means of controlling FMD in the future. Once identified as an FMD-free zone, this district can be one of the potential sources of livestock and livestock products for the country and for export.

Serological surveillance, in combination with clinical surveillance, is an accepted methodology to prove freedom from FMDV infection (OIE, 2009b). A statistically valid surveillance programme targeting all susceptible species is required in a country to confirm freedom from FMDV circulation (OIE, 2009a,b). In countries where FMD vaccination is routinely undertaken, such as in Bhutan, non-structural protein (NSP) tests should be used to monitor the circulation of FMDV in the susceptible population (Robiolo et al., 2006; OIE, 2009b). Clinical surveillance for FMD can be done directly through close physical inspection of animals for clinical signs (OIE, 2009b) or indirectly through interviewing of livestock owners (Bronsvoort et al., 2003). Serological surveys have been used in other countries to demonstrate freedom from FMD (Picão-Gonçalves et al., 2003; Isa, 2006).

Therefore, this study was conducted to validate the reported disease free status of the district (as determined by the current passive surveillance system) in order to pave the way for initiation of progressive zoning approaches to control FMD in Bhutan.

2. Materials and methods

2.1. Study area

Bhutan is a small landlocked country in the Himalayas sandwiched between China to the North and India to the South. Information about geophysical location and livestock husbandry system in Bhutan has been reported elsewhere (Dukpa et al., 2011a,b).

Tsirang district, with an area of 638.3 km^2, and altitude ranging from 400 to 2000 m.a.s.l., is located in the South-central part of Bhutan (http://www.tsirang.gov.bt/profile.php, accessed on 30th October 2009, Fig. 1). The district comprises 12 sub-districts and 65 villages. Livestock rearing forms an integral part of the overall agricultural production system through provision of draught power and farmyard manure. A national highway connecting the Southern border towns of Shompangkha and Gelephu to the capital city, Thimphu, passes through this district. The district supplies exotic cattle breeds to other districts. The district also has one of the highest small ruminant populations in the country (Anonymous, 2009a) and supplies sheep and goats to other parts of the country. Serological and clinical surveillance studies were conducted between March 2009 and August 2010.

2.2. Sampling strategy

For the purpose of this paper, villages were considered as the primary epidemiological units and the animals as secondary units.

2.2.1. First serological and clinical survey

For the first serological survey undertaken in March 2009, a two stage random sampling procedure was