



# Outbreak of Anthrax in central Bhutan, an environment-animal-human interface

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## Abstract

The outbreak of anthrax at Zhemgang district in Central Bhutan during 2010 caused death of 25 cattle; 8 horses; 4 pigs; 6 cats in 9 villages. The cattle got infected immediately after a heavy rainfall and 8 people developed severe cutaneous lesions on neck, fingers, arm and foot and all had history of contact with infected meat/animals. The animals had signs or symptoms of sudden death, bloated carcass, bleeding of unclotted blood from natural orifices. The disease was diagnosed by detection of gram positive bacilli and was further confirmed by bacterial isolation and molecular methods at Centre for Disease control (CDC) Atlanta, USA. The isolates were found to be part of the multilocus variable-number tandem repeat analysis B1 lineage (genotype 83) and canonical single-nucleotide polymorphism subgroup B.Br.001/002.

## Introduction

Anthrax is an acute infectious disease caused by bacteria *Bacillus anthracis*, which can affect almost all warm-blooded animals, including humans. In the environment, the bacterium rests as an endospore in the soil. Animals generally become infected through contact with soil-borne *B. anthracis* spores and humans usually become infected accidentally through contact with diseased animals or from handling the carcasses or by-products like skins and wool of diseased animals (WHO, 2008).

Outbreaks of anthrax have been reported to occur after heavy rainfall when anthrax spores get disturbed and brought to the surface (Anon, 2008). Mortalities in both animals and human have been reported in France during 1997 (Patra et al., 1998). In Bhutan the disease affecting both humans and animals was reported first time during September 2010, following a heavy rainfall in the Zhemgang district.

## Materials & Method

The multi-sectoral team comprising of representatives from National Centre for Animal Health, Department of Livestock, Ministry of Agriculture & Forests, Department of Public health, Ministry of Health investigated the outbreak. During the investigation, control program and also awareness program in school were conducted by the health counterpart. The information was collected during the awareness program. From the animal side, ring vaccination of the animals were carried out.

The data was collected during the investigation and also from the records from the animal health centers and Basic Health Units. The case of anthrax was suspected if an animal had signs or symptoms of infection like sudden death, bloated carcass, bleeding of unclotted blood from natural orifices; a case of anthrax was confirmed if rod-shaped bacilli were found by blood smear examination. The tissue samples from clinical cases were further confirmed by bacteriological and molecular methods at Centre for Disease control (CDC) Atlanta, USA. Eight variable number tandem repeat (VNTR) markers were used as described by Keim et al (2000) to analyze the genetic relationships.

## Results & Discussion

The first infection in cattle was reported in an animal being brought from another village. The animals started dying suddenly and the carcass were bloated and bleeding from nostrils (Fig. 1) were observed. Long dry period followed by heavy rainfall has been found to influence the outbreak of anthrax (Hassan et al., 2015) similarly, the outbreak at Kagtong village under Zhemgang district in the central Bhutan had occurred following a heavy rainfall in the area, where the top soil and vegetation have been washed by the rain water which would have exposed the anthrax spores. Though no isolation/identification of bacteria from the environment was done due to lack of facilities, the outbreak could be linked to the heavy rainfall since the disease was not reported from nearby places.

The disease spread was further aggravated by dressing of carcass and sharing of meat from the dead animal. The meat of the dead animals taken on horseback also led to infection in horse and also other animals of the herds where they had to take rest due to long journey. The disease spread as far on the top of the hillocks which is about 8 hours journey from the source of outbreak. 25 nos. of cattle, 8 horses/mules, 4 pigs and 6 cats had died due to anthrax (July 2nd week to 13th August 2010). The pigs were fed with the wastes of the meat and the cats were fed with the infected meat. This was mainly due to carrying of meat on horseback to another village and the farmer had about 37 animals; hence led to more cases.

The owner of the first case had dressed and sold the meat to his neighbors. Within 2-3 days, the skin lesions started developing. Some had small wound to even severe as swelling of entire arm. The lesions were present on the neck region, fingers, and arms, foot (Fig. 2) and even on the cheek; similar cutaneous cases were reported in other countries; more than 6000 cases in human was reported in Zimbabwe due to involvement in slaughter of infected cow during 1979 and 1980 (Turner, 1980) and cutaneous cases were encountered in Italy during 2005 (Kreidl et al., 2006).

*B. anthracis* was later isolated from three samples (two ear tip and one nasal swab) collected from three cattle from three villages (Thapa et al; 2016).



Fig. 1 Carcass -bleeding from nostrils.



Fig. 2 Cutaneous form of Anthrax on the leg

Isolates from the outbreak were found to be part of the multilocus variable-number tandem repeat analysis B1 lineage (genotype 83) and canonical single-nucleotide polymorphism subgroup B.Br.001/002 (Figure. 3). Unlike linkage A, B is found rarely and was found in South Africa and Europe (Van et al., 2007).

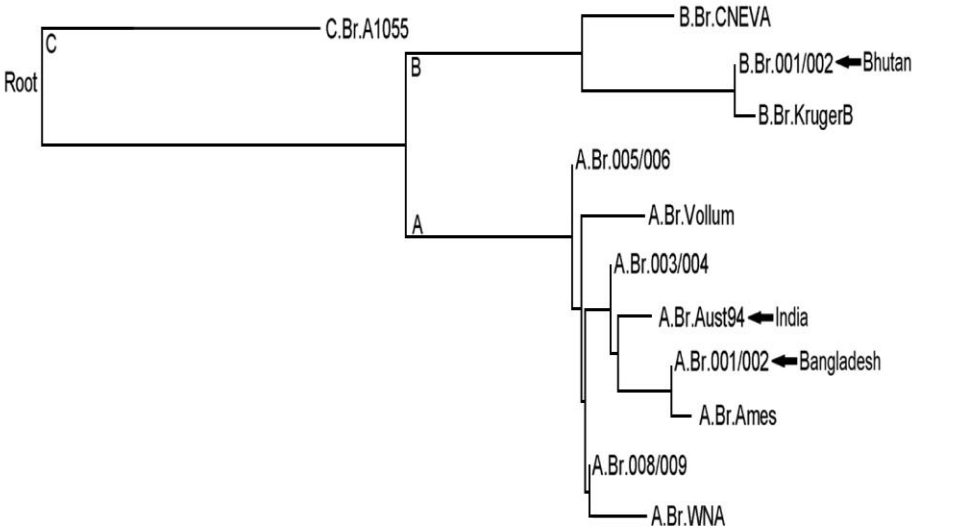


Fig. 3 . Phylogeny of major *Bacillus anthracis* groups as determined by using canonical single nucleotide polymorphisms; Thapa et al., 2015

To prevent the spread of outbreak, livestock and public health authorities carried out various prevention and control measures like creating awareness among villagers and students; ring vaccination of cattle against anthrax (≈445 animals in 11 villages); treatment of sick animals with antimicrobial drugs ; disposal of carcasses in deep burial pits; recall, collection, and disposal into burial pits of all potentially infected meat and hides from cattle that died of suspected or confirmed anthrax; and monitoring and treatment of persons in whom cutaneous anthrax developed. These control measures eventually contained the outbreak.

The outbreak was most likely linked to a heavy rainfall where the animals picked up infection and due to lack of awareness, the farmers got infected through dressing or handling of infected carcass.

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