A case of anthrax in wild elephant from the Western Ghats region of Kerala, India

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Anthrax is a highly contagious zoonotic disease which affects virtually all mammalian species (Quinn et al. 1994), caused by *Bacillus anthracis*, a Gram-positive, non-motile sporulating rod bacterium. It is a list B disease of the OIE (World Animal Health Organisation). The disease was one of the foremost causes of uncontrolled mortality in livestock worldwide during the late 19th and early 20th century. The development of effective livestock vaccine by Sterne, successful application of penicillin therapy and the implementation of quarantine regulations have drastically decreased livestock cases, but anthrax remains unchecked in wildlife due to the practical difficulties in vaccination (Hugh-Jones & De Vos 2002). Though the disease occurrence was reported in various species from different parts of our country, isolation and identification of *Bacillus anthracis* from wild elephants has not been well documented in India and has not been reported in Kerala.

On 29 September 2006, the death of a wild elephant was reported by a forest guard to the local veterinary surgeon. The animal belonged to the Chedleth forest range, Pulpally of Wayanad district of Kerala, which is a border area between the two states, Kerala and Karnataka. A detailed examination of the carcass revealed discharge of blood from the eyes, trunk and also from a deep cut wound in the middle part of the trunk. On suspicion of anthrax, the veterinary surgeon examined peripheral blood smears prepared from the cut wound as well as from the eyes. The duplicate smears were sent to District Veterinary Centre, Kalpetta. No pathogenic organisms could be detected on any of the stained smears by Leishman's technique. Hence, a post-mortem was conducted; no abnormality could be detected except for the unclotted blood from the internal organs. For detailed laboratory examination, heart blood was brought to the Department of Veterinary Microbiology. Thin blood smears were prepared and stained by Leishman's stain and examined microscopically. Heart blood was cultured on nutrient agar and the plate was incubated aerobically at 37^{o}C for 24 hours. The pure culture obtained was characterized and the isolate was confirmed as *Bacillus anthracis* by mice inoculation test as per the method described by Lennette (1980).

Blood smear stained with Leishman's stain revealed numerous blue rods with typical truncated ends in short chains. (Image 1). For the demonstration of capsule, polychrome methylene blue staining was done. It revealed blue colored bacilli with pink colored capsular material, the 'Mac Fadyean reaction', which is considered as pathognomonic for anthrax (Quinn et al. 1994).

On nutrient agar, irregularly round colonies of about 4mm in diameter, flat, dull, opaque, greyish-white and a frosted glass appearance were noticed. Under low magnification, the edges of the colonies resembled locks of matted hairs, the typical 'medusa head appearance'. The colonies were weakly haemolytic on blood agar. Gram's stained culture smear revealed Gram positive bacilli arranged end to end in long chains and presented a 'bamboo stick' appearance.

An inoculum for animal pathogenicity testing was prepared by scraping some growth from an 18-24 hr incubated agar plate with a loop and emulsifying into 10ml of sterile saline in a test tube by holding the tube in a 45^{o} angle and rubbing some growth into the area of meniscus so that the upper 0.5ml of saline

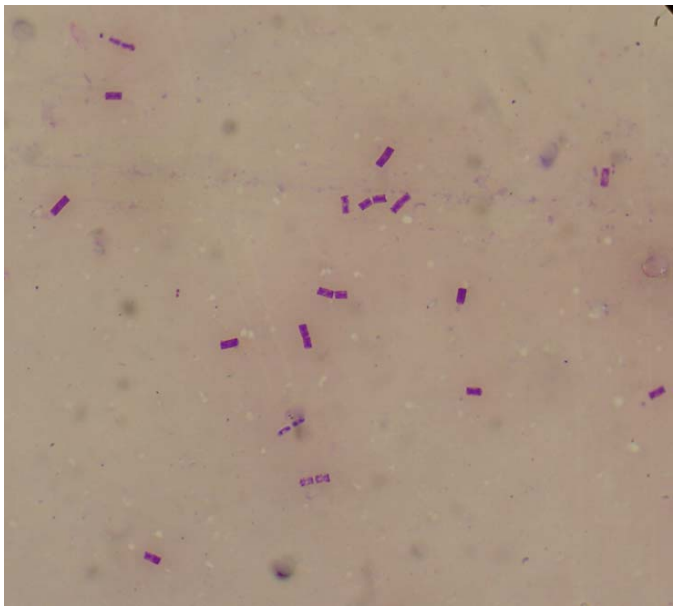

Image 1. Blood smear from a wild elephant with anthrax. Numerous large truncated-end rods typical of *B. anthracis* are present in the smear. Leishman's stain

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became slightly turbid. Tube contents were mixed and the resulting suspension did not show any turbidity (contained approximately 10^5 to 10^6 organisms/ml). Two mice were each inoculated with 0.1-0.2ml subcutaneously and one mouse kept as control. The animals were observed at 6-hourly intervals and the test mouse was found to be dead 30 hr of post inoculation. The post mortem conducted on the dead mouse revealed hemorrhagic lungs, liver and splenomegaly. Impression smears from liver, lungs, spleen and heart blood smear readily revealed the bacilli by Leishman's staining. The inoculated organism was re-isolated from all the organs with lesions, thus proving the Koch postulates.

Control measures like precautionary disposal of carcass, public awareness programs and vaccination of animals were successfully done by the veterinary officials, and the forest officials were also informed.

According to Hugh-Jones & De Vos (2002), variation in the number of bacilli in a peripheral smear could be expected not only within the animal species, but also between species. Some animals show consistently high terminal bacteraemic counts, while in others few or no *Bacillus anthracis* organisms could be detected terminally. Low numbers could also be expected in animals treated with antibiotics or in those possessing some immunity. In the present case, the animal did not have enough organisms to be detected in peripheral blood smears. This forms an interesting and informative case of anthrax in a wild elephant in Kerala.

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