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RAPID REPORTS AND PERSPECTIVES FROM THE FIELD

The first laboratory-confirmation of anthrax during an outbreak in Guinea

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Abstract

The prefecture of Koubia in the Labé region of Guinea suffers from permanent threats due to anthrax outbreaks. However, investigations have never benefited from laboratory diagnostic testing and have relied only on clinical diagnosis. The most recent anthrax outbreak in Guinea occurred in February and May of 2019 in Koubia, and involved two different communities. In this work, we performed laboratory diagnostic testing on samples collected during field investigation. We report the first evidence of laboratory confirmation of anthrax in Guinea. This work highlights the importance for conducting study to understand the dynamic of anthrax at the human, animal, and environment interface in the prefecture of Koubia.

Key words: Guinea, Koubia, anthrax, laboratory, diagnostic.

Bacillus anthracis is the causal agent of anthrax, a zoonotic disease that threatens public health. It has previously been used as biological weapon and is classified as a biological threat [1-3]. *Bacillus anthracis* spores are highly resistant to extreme conditions and can remain viable in the soil and in animal products for years. Infection of humans occurs by exposure to contaminated animals and animal products, breathing of spores, or introduction of spores at a site of skin cut [4].

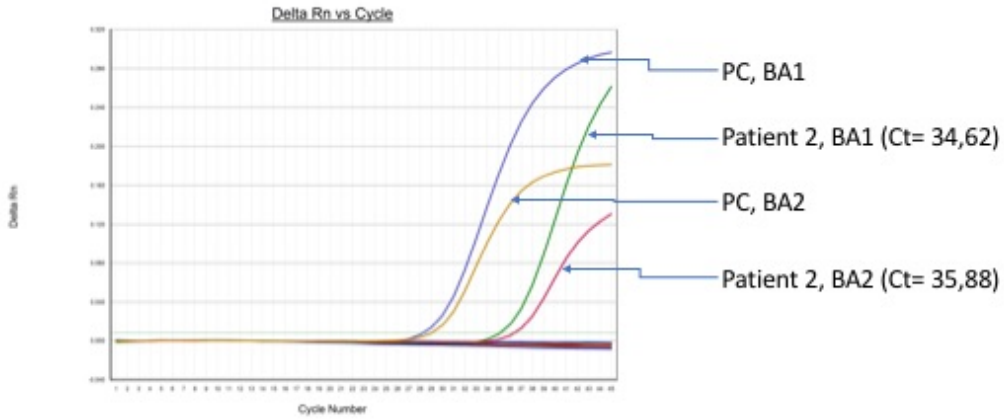
The prefecture of Koubia, Labé region of Guinea has long been threatened by multiple and sporadic anthrax outbreaks. During the 2014 anthrax outbreak in Koubia, epidemiological, therapeutic, and clinical features were described during an outbreak of 39 cases [5]. However, laboratory investigations and confirmation of the etiologic agent of the outbreaks has never been carried out mainly because of the lack of resources for laboratory diagnostics. The need for laboratory investigation to differentiate infection due to *Bacillus anthracis* from other infections is substantiated by the fact that in the region, other prevalent diseases may present with similar clinical symptoms. With the Guinea country laboratory capacity building and strengthening after the Ebola outbreak, resources and capacity for anthrax laboratory diagnostic testing were developed at the National Institute of Public Health. In February and May 2019, two episodic outbreaks of mostly cutaneous anthrax occurred in the prefecture of Koubia. There were 3 cases in February and 52 suspected cases in May 2019. Investigations were conducted by a team of health workers and epidemiologists and whole blood samples were collected from suspicious cases and sent to the laboratory at the National Institute of Public Health for testing. A total of seven samples (3 in February and 4 in May) were received at the laboratory. For the outbreak that occurred in May, a sample could not be obtained from one patient because he had died. All samples were collected before initiation of antibiotic treatment. DNA was extracted from 50 µl of each blood sample by using the RTP pathogen kit (Stratec Molecular GmbH, Germany) and following the manufacturer's recommendations. The presence of *Bacillus anthracis* was detected by real time polymerase chain reaction (rtPCR) using BA1 and BA2 reagent mixes, enzyme Taq platinium and positive controls BA1 and BA2 obtained from the Defense Biological Product Assurance Office (DBPAO). Primers and probes target regions of the pXO1 and pXO2 plasmids carried by the *B. anthracis*. Sample quality was assessed by amplifying a fragment of the ribonuclease P gene. Amplification reactions were run on the ABI 7500 Fast Dx Real Time PCR instrument (Applied Biosystems, US) and analyzed using the Applied Biosystems 21 CFR Part 11 Module 7500 Fast Dx Real-Time PCR System sequence detection software version 1.4 (Applied Biosystems, USA).

Suspected cases most likely resulted from consumption by family members of goat meat from sickened and slaughtered animals. Clinical symptoms appeared within 5 to 12 days post exposure and are summarized in the **table**. From the three samples received during the February outbreak, one sample was tested positive (**Figure**) leading to a positivity rate of

33.3%. The positive case was a female student aged 8 years old. All four samples received during the outbreak that occurred in May tested negative.

This report provides the first laboratory evidence of anthrax in Guinea and highlights the need for laboratory investigation during future outbreaks. It warrants the need for continuous surveillance in the region and underscores the need for investigational study to understand the root causes of the anthrax outbreak endemicity in the Koubia prefecture of Guinea.

Figure 1. Molecular detection of *Bacillus anthracis*.



PC: Positive control; BA1: *Bacillus anthracis* target 1; BA2: *Bacillus anthracis* target 2; Ct: threshold cycle.

Table 1. Socio-demographic characteristics and clinical symptoms of the patients.

Patient ID	Residence	Sex	Age (year)	Profession	Clinical symptoms	Laboratory result
Patient 1	Koubia	M	10	Student	Fever, sore throat, black scab	Negative
Patient 2	Koubia	F	8	Student	Fever, nausea, black scab	Positive
Patient 3	Koubia	M	14	Student	Fever, nausea, sore throat, fatigue, headache, fainting, red eyes, confusion, black scab	Negative
Patient 4	Koubia	M	9	Student	Skin lesions, black scab, pruritus	Negative
Patient 5	Koubia	F	21	Housewife	Skin lesions, pain, difficulty breathing, abdominal pain, vesicle	Negative
Patient 6	Koubia	M	9	Farmer	Skin lesions, generalized pain, abdominal pain, sore throat, black scab, fatigue, neck stiffness	Negative
Patient 7	Koubia	M	2	-	Fever, fatigue	Negative

Competing interests
The authors declare that they have no conflict of interest.

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Authors' contributions
MBK, JN, RC, and AT conceived the study; BT, PT, MBK, JN, SM, MK, JT performed the experiments; JN, MBK, and AT wrote the manuscript. All authors read and approved the manuscript.

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. By the Republic of Guinea law, this study did not require approval by the health research ethic committee because it was an investigational study for disease surveillance. The investigation was approved by the Ministry of Health and Hygiene as part of the surveillance of diseases with epidemic potential.

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