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Clinical-molecular study of anthrax in the prefectures of Kankan and Koubia (Republic of Guinea)

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Abstract
Anthrax has been suspected by breeders, auxiliaries, livestock workers and veterinarians in the field. At the end of this suspicion and the recent outbreaks in 2017 in Kankan and Koubia causing deaths and victims among the animal and human populations, 26 soil samples and 14 bones from the cursed fields were carried out in two rural communes of administrative regions of Kankan and Labé in the prefecture of Koubia. Analysis of these samples confirmed that of the 27 soil samples taken, 5 are positive, including 3 in Koubia and 2 in Kankan.

Keywords: Present status, Problem confrontation, Oilseed cultivation

1. Introduction

Anthrax is a worldwide zoonotic disease caused by *Bacillus anthracis*. It affects mammals and a few species of birds, but it is the herbivores that are generally the most affected. It is to be distinguished from the symptomatic anthrax that caused by *Clostridium chauvei*. Listed on the list of the World Organization for Animal Health, it is one of the diseases known to be contagious in France and gives rise to the application of animal health measures. It is also recognized as an occupational disease and has become, since 2001, a notifiable disease in humans. Anthrax is a serious zoonosis of worldwide distribution caused by a spore-forming "telluric" bacterium, *Bacillus anthracis* (OIE, 2008) [1].

The extensive breeding practiced in the country, the zoosanitary conditions, the climate and the nature of the Kankan soils in Upper Guinea and Koubia (Middle Guinea) are very favorable factors for the spread of anthrax, sporulation and conservation of the spore. This situation explains the existence of the cursed fields which are largely at the origin of clinical cases of anthrax in humans and animals. In addition to its sporulation capacity, *B. anthracis* has several virulence factors coded by genes located on its two plasmids (pXO1 and pXO2): a capsule with anti-phagocytic properties and two type AB exotoxins, the Edematogenic Factor (EF) and the Lethal factor (LF), sharing the same factor ensuring the translocation through cell membranes to the cytosol of the two other factors, factor PA (Protector Antigen) (OIE, 2008). Anthrax in Guinea is very worrying because the disease appears endemic each year in Middle Guinea and Upper Guinea and sporadically in Lower and Forest Guinea. The disease frequently strikes cattle, sheep and goats and rarely humans.

Over the past two decades (1995-2016), one thousand ninety-three outbreaks (1093) of anthrax have been identified. The number of outbreaks reported each year is extremely variable, ranging from poorly active years to years conducive to the resurgence of anthrax cases. There were 19 outbreaks in 2013 and 27 outbreaks with 17 human cases including 10 dead in Koubia and 12 patients for 7 dead in Kérouané in 2014. The persistence of anthrax outbreaks in Guinea and in the West African sub-region can be explained by a number of factors: factors specific to the pathogen (telluric bacteria), climatic factors as well as socio-economic and cultural factors.

The morbidity and mortality rates recorded in Guinea vary from year to year according to the different species with a lethality which varies between 99% in 2007 to 32% in 2015 in cattle and 100% in small ruminants for the same year. Anthrax is a hydro-telluric reservoir disease. Sick animals, their corpses, their secretions, their excretions and the contaminated soil constitute the sources of bacteria. Most often, transmission occurs indirectly through ingestion of
water or food contaminated with contaminated soil or food prepared from contaminated raw materials. Transmission, through bites (by blood-sucking insects or contaminated objects) or following contamination of a wound, is possible [2]. Herbivores and, in particular, ruminants are the most exposed to anthrax. The disease is most prevalent in summer in grazing cattle that ingest soiled grass. The incubation period lasts an average of 4 to 8 days [3]. There are acute, subacute and super acute forms of the disease.

In the acute form, called septicemia anthrax, cattle have sudden onset of general condition with chills, hyperthermia at 41 - 42°C and cessation of milk production. In 12 to 24 hours, respiratory and circulatory disorders appear: there is

tachycardia, dyspnea and the mucous membranes become congested then cyanotic. Cattle sometimes show digestive signs including colic, bloody diarrhea, footprints and tenesmus. Urinary signs, such as hematuria, appear later. Nervous signs, such as muscle tremors or convulsions, are sometimes observed. Death occurs in 2 to 3 days.

In the acute form, cattle show similar but more severe signs. Death occurs in 6 to 12 hours. Sometimes death occurs, more suddenly, without clinical warning signs. In the subacute form, the general signs are identical to those of the acute form but there is, in addition, an external swelling. Death occurs in 4 to 5 days [4].

Skin signs can be seen in subacute forms of the disease, called external forms or tumor coals. Cattle show an edematous reaction, reaching 20 to 30 cm in diameter, within a few hours. It is a warm, painful, non-crackling swelling, most commonly located in the throat area or at the entrance to the chest. In some cases, necrosis of the skin, giving rise to erosions or ulcers, can be observed in the thoracic region, without any other signs of disease.

Epidemiological and clinical elements allow the practitioner to suspect the disease. In addition, the autopsy, which should not be performed in the field, reveals lesions characteristic of anthrax: the blood is thick, sticky, blackish and uncoagulable; the spleen is enlarged, flaccid, blackish and muddy in consistency; the bladder contains bloody urine. There is frequently an absence of cadaveric rigidity. Confirmation of the diagnosis is based on laboratory evidence of *Bacillus anthracis* in the blood, spleen, liver, lungs or long bones [5].

The prognosis is generally grim, mostsick animals die. Treatment is based on antibiotic therapy. Many molecules are effective, but penicillin is the antibiotic of choice. The effectiveness of the treatment is conditioned by the precocity of its implementation. Managing anthrax involves transporting dead animals to rendering, destruction of litter by fire, cleaning and disinfecting equipment and contaminated areas. Prevention of the disease is based on the use of pastures known to be contaminated. In contaminated areas, medical prophylaxis is necessary and is based on the use of live attenuated vaccines [6, 7].

This zoonosis causes heavy economic losses to the ruminants of our country by the high mortality of animals with sometimes loss of human life. To this end, it represents a great threat to public health.

2. Material and methods

2.1 Material

This research focused on certain localities in the prefectures of Kankan (Kankan Koura district) and Koubia (Dayan and Katia Linguere). These two prefectures are located in Upper Guinea and Middle Guinea respectively.

The material for collecting clinical and post-mortem informations is made up of survey sheets. The technical material for sampling from a corpse consists of: sampling kit (1), tubes (65), sampling rod (QS), and personal protective equipment (6), boots (2), latex or rubber gloves (QS), Helmets (2), Protective goggles (2), 70 ° ethyl ether and alcohol swab on sterile tape (QS), muffler (QS), plastic bags (QS) and plastic tie belts (QS). Biological material consists of long bones collected from the corpses of domestic animals and the soil infected by sick animals.

The laboratory equipment for real-time PCR consists of: PCR reader (Rotor Gene 6000), Oven, Ultra Centrifuge, Micro tubes, Consumables (Primers, Taq polymerase, nucleotides, etc.), Premix: (two hundred and eighty milliliters (280 ml) of distilled water are mixed with 100 µl of the PCR buffer; 1.7 µl of 100 mM of each of the oxyribonucleotides (dATP, dCTP, dGTP, dTTP) and 5 µl of primers of 100 nmol / ml and DNA solution to be studied.

2. Methods

We have had suspicions during ordinary visits to farms or when the technical agents have been called by breeders. Clinical suspicions were made by network agents and according to the surveillance guide or protocol and were noted on a suspicion sheet which contains information on the locality, the species affected, the transhumance axis when it occurs acts of transhumant, the numbers of animals from an affected farm, the number of dead and sick, symptoms and lesions observed by the agent or reported by the farmer concerned, as well as information on watering points and hematophagous insects present in the locality [8, 9].

The sampling focused on the cursed fields previously identified by the agents of the Animal Disease Network in Guinea. To do this, we used a sample card which contains information on the identification of the place, the breeder, the affected species and especially the identification of the animal sampled (age, sex, symptoms or lesions observed as well as the nature of the samples taken).

The sampling operations consisted in wearing personal protective equipment), weeding and cleaning the sampling area, subdividing the area into a square of 4 m long and 4 m wide, identifying the sampling sites (the 4 sides of the square and the medium), take, depending on the different targeted zones (Surface, medium and depth), 100 to 200 g of soil in a test tube by coring every 30 cm from the field and some long bones in depth if they exist, label- the samples and return them to the sample box and transport the samples as quickly as possible to the laboratory for analysis. We used the “DNA / RNA RIBO-prep” protocol of AmpliSens OKI Screening-FL. »Of the PCR method for DNA extraction [10, 11, 12].

3. Results and Discussion

3.1 Results

The results of the detection in the different sampling zones are given in Table 1.

Table 1: Results of laboratory analyzes

Dated	Sampling site	Sample		Number	Results			Method
		Nature	Type		Positive	Negative	Not studied	
04/11/2017	Kankan (Kankan Koura)	Ground	Area	4	0	4	0	PCR -ADN-Bacillus anthracis
			Middle	5	2	3	0	
			Depth	5	0	5	0	
		Bone	Long bone	6	0	0	6	
	Subtotal 1			20	2	12	6	
13/11/2017	Koubia (Dayan)	Ground	Area	2	0	2	0	PCR-ADN-Bacillus anthracis
			Middle	2	0	2	0	
			Depth	3	3	0	0	
		Bone	Long bone	4	0	0	4	
	Subtotal 2			11	3	4	4	
14/11/2017	Koubia (Katia Linguéré)	Ground	Area	1	0	1	0	
			Middle	2	0	2	0	
			Depth	2	0	2	0	
		Bone	Long bone	4	0	0	4	
	Subtotal 3			9	0	5	4	
	Total			40	5	21	14	

In Table 2, only the results obtained from the samples taken in the field in the cursed fields are given.

Table 2: Results obtained from samples taken from cursed fields

Sampling location	Number of soil samples taken	Confirmation or Positive	No confirmation or Negative (%)
Koubia (Dayan)	7	3 (16,66 %)	4 (33,33 %)
Koubia (Linguéré-Katia)	5	0 (0 %)	5 (41,66 %)
Kankan (Kankan Koura)	14	2 (14,28 %)	12 (85,71 %)
Total	26	5	21

3.2 Discussion

The table 1 shows that out of the 26 soil samples taken, 5 are positive, including 3 in Koubia (Dayan) and 2 in Kankan (Kankan Koura). The existence of *Bacillus anthracis* in the substrates of the external environment would be explained by the favorable climatic conditions in these areas (heavy rainfall in the rainy season and drought in the dry season), the nature of the soils (sandy and ferritic), by the presence of termites and the occurrence floods in most affected pastures. These conditions undoubtedly favored the return of bacterial spores present in the soil. Also, the germs were found in Kankan Koura in the middle while in

Dayan they were found in depth. The rise of the spores from the depth to the surface would probably due to the nature of the soils, the presence of termites in these zones, the number of years spent after burial, the methods of incineration and burial applied.

While the back samples sent to the laboratory could not be analyzed for technical and safety reasons. And yet the probability of finding *B. anthracis* on these non-analyzed samples is very high since they were taken from cursed fields.

Starting from Table 2, we note that these laboratory diagnoses essentially aimed at confirming the samples taken in the field in the cursed fields. All these positive samples came from corpses buried since 2014. The soil samples at Koubia (Dayan) revealed 3 positives out of the 12 samples taken while those from Kankan (Kankan Koura) gave only 2 positives out of the 14 samples taken. This shows that the spores of *Bacillus anthracis* have remained for 3 years in these areas and if all the health and safety measures are not taken to limit them, they will always constitute a permanent health threat of the grazing ruminants as well as that of the population living in these localities.

4. Conclusion

The type of extensive breeding practiced, the zoo sanitary conditions, the climate and the nature of the Kankan soils in Upper Guinea and Koubia (Middle Guinea) are very favorable factors for the spread of anthrax, sporulation and the conservation of the anthrax spore. This situation explains the existence of the cursed fields which are largely at the origin of clinical cases of anthrax in humans and animals. Anthrax in Guinea is very worrying because the disease appears endemic each year in Middle Guinea and Upper Guinea and sporadically in Lower and Forest Guinea. The disease frequently strikes cattle, sheep and goats and rarely humans.

The risk of human infection is established during the slaughter of sick animals and the consumption of meat from infected animals and this constitutes a socio-cultural practice generally motivated by ignorance and financial needs to preserve the livelihoods of the farmers. This problem must be addressed by effective community approaches and incentives to convince rural communities not to slaughter sick animals and not to eat their meat.

The fight against anthrax requires intersectoral coordination between veterinary and public health authorities, particularly in the field, through a structured exchange of information, joint investigations of suspected cases and better coordination of the implementation of interventions. The fight against anthrax provides an ideal opportunity to operationalize the "One Health" concept.

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