

RE-EMERGENCE OF PLEUROPNEUMONIA CONTAGIOUS BOVINE IN THE GAMBIA

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ABSTRACT

The authors mentioned the epizootic infection for Contagious Bovine Pleuro Pneumoniae (CBPP) caused by *Mycoplasma mycoides subsp. mycoides* biotype Small Colony (*MmmSC*) which has affected Ndama bovine in Niamina Dankunku a locality based in Abuko city in the eastern part of the Gambia during the rainy season in 2012. After the cessation of vaccination a hotbed of suspicion of CBPP was identified on August 3rd, 2012 in the town of Niamina Dankunku. Thus, with a total of 400 cattle including 18 patients in whom 12 died. These studies have been done according to clinical aspects, serological and bacteriological analysis of the samples. This re-emergent disease in Gambia gives new orientations about CBPP in Senegal where it has to be eradicated since 2005.

Keywords: Pleuro pneumoniae - *Mycoplasma mycoides subsp. mycoides* Small Colony (*MmmSC*) – Bovine – Niamina Dankunku – Re emergent disease – Gambia - Senegal

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INTRODUCTION

CBPP is an infectious, contagious, communicable disease, caused by *Mycoplasma mycoides subsp. mycoides biotype Small Colony (MmmSC)* [15, 21]. It is characterized clinically by respiratory (cough, dyspnea, nasal discharge), joint disorders (lameness) among young bovine under two years and on the lesions of pneumonia and exsudative pleurisy, sero-fibrinous in acute cases and the presence of pulmonary sequestration in chronic cases [4, 10].

It mainly affects cattle (*Bos indicus*, *Bos taurus*) and water buffalo (*Bubalus bubalus*). Because of its economic importance, this disease belongs to the list of priority diseases in the world as defined by the World Organization for Animal Health (OIE) [20]. CBPP has been eradicated in many countries in Europe, North America Sub-Saharan Africa in the 20th century according to the application of good health policy. Gambia stopped vaccinating for forty years and Senegal since 2005 is in the position of eradication of the disease [12, 16, 17]. CBPP is transmitted by direct contact between infected and healthy animals. The presence of CBPP in suspect animal can be confirmed in the laboratory by the direct method by isolation and identification of the causative agent from suspicious lesions or by the indirect method consisting highlighting the possible presence *anti-MmmSC* antibodies. In view of its eradication, a monitoring of the disease is established in Senegal since 1986 [9] and based on the sero-epidemiology and Senegal has stopped vaccination since 2005. CBPP were so rampant endemic for several years in tropical Africa and has induced important losses which are difficult to assess because of its insidious aspects. In Africa, the disease control is based on vaccination campaigns using attenuated strains such as T1/44 or T1sr. Unfortunately, efforts to control relaxation experienced causing an outbreak of cases are very difficult. Otherwise, In Botswana, a major outbreak of CBPP was reported in 1998 [1]. In Gambia, the last case of contagious bovine pleuro pneumonia went back in 1971 and since then, no outbreak has been reported [20]. Vaccination against the disease has been stopped in 1987. For this, according to the epidemiological surveillance system put in place after the cessation of vaccination a hotbed of suspicion of CBPP was identified on 12 - 08 in the town of Niamina Dankunku.: Thus with a total of 400 cattle with 18 patients in whom 12 died. Identification techniques have been described for *Mycoplasma* everywhere. [2] Other methods of identification have used like immuno fluorescence techniques as well as the DNA staining methods using DAPI [13]. Other methods have also been used the DNA hybridization using specific probe rRNA mycoplasmal [19]. The serological method for detecting *Mycoplasma mmSC* antibodies by Technical Enzyme Linked Immuno Sorbent Assay (ELISA) using specific monoclonal antibodies proved to be very sensitive [8]. [13]. We have used indirect methods (immuno - serology) and direct methods for isolation and identification of the causative agent *Mycoplasma mycoides subsp. mycoides biotype Small Colony (MmmSC)* from samples of suspected blood and parts of the lungs thereby presenting lesions. The re-emergence of this disease in the Gambia can induce the problem of its risk for extension to one of the neighboring countries, Senegal, where the disease is in its eradication phase [12, 16, 17]. It is thus urgent to take necessary and useful for enhanced control of the disease in these two neighboring countries.

MATERIALS AND METHODS

Location and description of the town Niamina Dankunku : The focus of suspicion of CBPP appeared on 12 - 08 in the town of Niamina Dankunku Gambia (see in Figure 1). The administrative situation for Niamina Dankunku based is in the eastern part of The Gambia: (see in Figure 2) : Niamina East is one of the ten districts of the Central river division of the Gambia. Niamina Dankunku is at 2 kilometers east from Wellingara Ello locality.

1. Bacteriology: Isolation: After grinding in a broth made with meat extract (beef heart infusion) supplemented with peptone and yeast extract beer 10% final concentration serum, this medium should contain glucose (2g / l), phosphate (buffer system), glycerol and DNA but also antibiotics bacterial binding inhibitors such as penicillin G (100UI / ml), the Thallium acetate (1/10000) and fungizone in order to avoid fungal contamination. Environments are used either liquid or solid (by adding agar or agarose (15g / l). Liquid media were sterilized by filtration and solid media are autoclaved. To perform isolation, lung samples were diluted from 10 to 10 to minimize bacterial contamination and are inoculated into tubes of broth and on solid medium for 200µl. Blood was inoculated directly without dilution. Incubation is carried out with the presence of buffer Gaspack for microaerophilic, in a bell and the whole set in an incubator set at 37 °C. Petri dishes and tubes are examined daily for 10 days. **Identification:** Conduct a preliminary purification of the colony observed by performing three consecutive purifications cloning. In cases where the broth is contaminated, we have used filter disk with 0,5porosity. **Biochemical characteristics:** We have sown the seeds of the pure culture in the media (Glucose, Arginine, Phosphate) and incubated in an incubator at 37 °C for 24 hours. Final identification is done by using serological means like the competitive ELISA Test. This study involved four bacteriological samples from Niamina1, Niamina 2 Basset 1 and Basset

2. Serology and Immunology: The enzyme linked immunosorbent assay (ELISA) was used for competition: This implementation method for research CBPP antibodies is the one recommended test by CIRAD Emvt Centre for OIE Reference for Contagious Bovine pleuropneumonia (CBPP). Competitive ELISA is a technique used by CIRAD Kit / CBPP - competitive ELISA, Version P05410/01- / 06/2006 [14] and has been chosen for its high sensitivity. This technique has been approved by the OIE like the Complement Fixation Test (CFT) based on the technical method done by TURNER and CAMPBELL (CAMPBELL and TURNER, 1953) [5]. It was also developed by CIRAD: CIRAD / RFC - CBPP - ref 02230085 (Dannacher & (G.) *et al*, 1986). [6]. This study concerned 18 serological samples from Niamina 1 locality in total.

RESULTS:

Clinical diagnosis: It was found pulmonary lesions with characteristics petichies for the four samples received (see in fig 1). **Bacteriology:** On solid medium: after four days of incubation, we have observed from the sample Niamina 1, characteristic colonies like "in fried egg" 1mm for size has been observed under the microscope. These colonies showed centers embedded in agar which have been verified by the agar is still torn after collection. Microscopic observation in immersion after Giemsa staining showed that these elements have a size which is near 1mm, colored purple (not bacteria none viruses) On liquid medium: After four days of incubation we observed from the culture obtained on Niamina 1 sample, some mild and consistent liquid with a uniform opacity producing more fleeting waves shimmering in agitation. The medium which was initially red turned in yellow confirming its use by the bacteria. The fresh made from the medium, observed through magnification from the resulting culture of the Niamina 1 sample, showed very small particles which are not bacilli or cocci,

smaller than that of bacteria and made up in small long filaments slender points and agitated by a strong brownian motion. The study of biochemical characteristics gave the following results namely the hydrolysis of glucose, arginine and phosphate from the culture obtained on Niamina 1 sample which are characteristic for *Mycoplasma*. The results obtained for samples from Basset 1, Basset 2 and Niamina 2 were negative by lack of culture on both solid and liquid medium

Seroimmunology: The results obtained after reading optical densities and the calculation of inhibition percentages with the positivity threshold is around 50% are reported in Table I: We can say that the following animals with PI% > or equal to 50 (Nos. 1, 3, 4, 5, 6, 7, 8, 12, 13, 14, and 16) positives, are carriers of specific antibodies *mycoides subspecies mycoides* ANTI *Mycoplamsa SMALL COLONY (MmmSC)*, the agent of contagious bovine pleuro pneumonia (CBPP) with a very high prevalence of 66% in the actual analysis in this locality Niamina Dankunku.

Figure 1: Gambia Map : Localisation of Niamina Dankunku in Gambia (Eastern part of the Gambia)

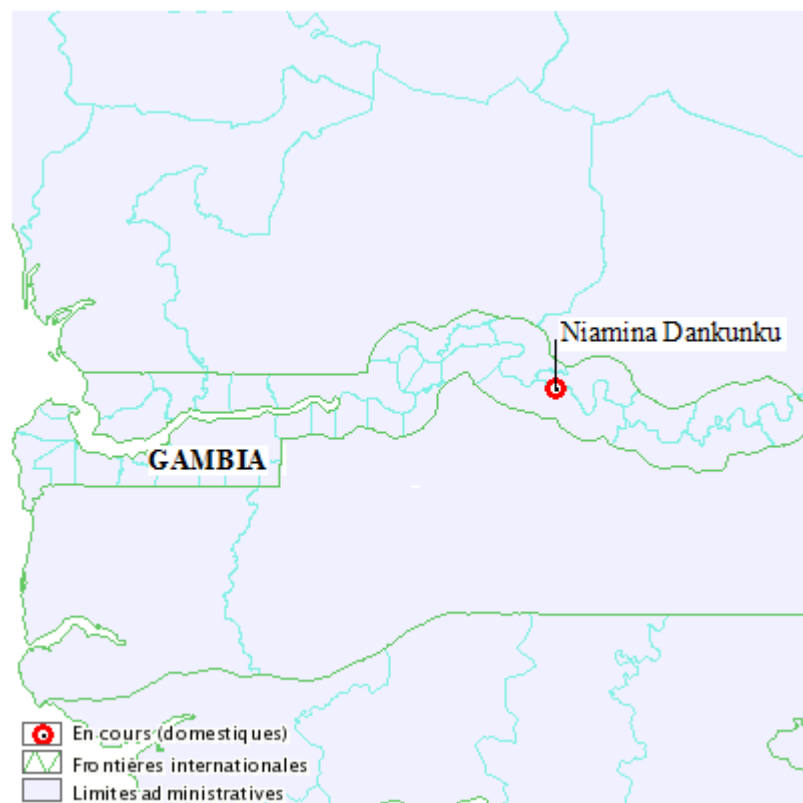


Figure 2: Administrative situation for Niamina Dankunku in the Gambia.



Figure 3: Ndama bovine from Niamina Dankunku taking water drinks in the local river.



Table 1: Competitive ELISA Serological results from 19 suspicious Ndama bovine serums in the locality of Niamina DANKUNKU - Gambia

Animal Numbers ^o	OD	IP %	Résults	Animal Numbers	OD	IP %	Résults
1	0,453	59	Positive	11	0,655	37	Négative
2	0,809	21	Négative	12	0,435	60	Positive
3	0,401	64	Positive	13	0,391	65	Positive
4	0,377	66	Positive	14	0,497	53	Positive
5	0,504	52	Positive	15	1,093	0	Négative
6	0,533	50	Positive	16	0,469	56	Positive
7	0,366	67	Positive	17	0,669	35	Négative
8	0,304	74	Positive	18	0,763	26	Négative
9	0,859	15	Négative	19	1,573	0	Négative
10	0,656	37	Négative				

OD: Optical density read at λ 450 nm

IP% : Inhibition Percentage

DISCUSSION:

CBPP is a re-emerging disease in the Gambia according to the results obtained. Transhumance and movement of animal and people in recent years have the opportunity to demonstrate that infection is a serious obstacle to the development in our countries. The annual vaccination campaigns followed the same sero epidemiological must be strengthened to prevent this disease. It is imperative to ensure the implementation of prevention tools that who fear infection cause huge economic losses in Gambia or in Senegal and also in West Africa. This disease in neighboring countries showed relatively high rates in Mali as said by Bashiruddin B JB (1996) and in Mauritania [3] and thus constitutes a serious threat to livestock development in the sub region including Gambia. This high prevalence of the disease in the Gambia could towards the establishment of a serious epidemiological surveillance system internally and in neighboring countries such as Mali, where the disease is around 4 to 10% and mortality for 3 with 8% in enzootic area, 25% of chronically infected animals after treatment in the same way that the unapparent infection remain dangerous sources of infection long term factors favoring the spread of the disease in Mali is the extensive nature of the livestock, transhumance and antibiotics [3]. In Guinea, the prevalence of individual CBPP equal to 6.3% is high [7]. The effects of age and sex and race are real on the prevalence of CBPP. In our study it is mainly aged females Ndama which are the main carriers of antibodies to *Mycoplasma mycoides subsp. mycoides* in Gambian territory : this

corroborates the observations made in Mali by Bashiruddin JB whereby, that old female zebu Bos indicus cattle breed are able to keep their long antibodies, with the risk to be chronic carriers which will be dangerous for the transmission of CBPP, unlike calves [3].

CONCLUSION:

Although CBPP outbreaks seem to have disappeared from the Gambia for forty years, when carrying anti-CBPP revealed by this study from the serological test competitive ELISA and carrying germs revealed by conventional isolation and identification could be compatible with a situation of recent contamination by *Mycoplasma mycoides subsp mycoides SC* and translated as a possible resurgence of the disease in the Gambia. It is therefore necessary to conduct Gambia and neighboring countries for a wider sero-epidemiological surveys to locate any new outbreaks of CBPP and analyze the importance for risk factors. Gambia and Senegal have to combine their efforts in order to develop new strategies for stopping this diseases especially at the trans boundaries areas where the movement of cattle are more frequent.

REFERENCES

- 1 - Amanfu W. MASUPU KV, ADON E K. Raborokgwe MV BASHRUDDIN J, B. - An outbreak of contagious bovine pleuropneumonia in Ngamiland district of North - Western Botswana - Vet. Res. 1998.
- 2 - BARILE MF., Rottem S (1993) - *Mycoplasma* in cell culture: In Kahan, Adoni A (eds) - Rapid diagnosis of *Mycoplasma* - Plenum Press, New York, pp 155-193.
- 3 - Bashiruddin J.B. (1996). Observations from Outbreaks of CBPP in Europe and Africa. In: (J. Frey, K. Sarris, editors) *Mycoplasmas of ruminants: pathogenicity, diagnostics, epidemiology and molecular genetics, and biotechnology Agriculture*, Berne, p.150-154.
- 4 - CHANTAL J. - Contagious bovine pleuropneumonia. EISMV lecture. Dakar 1976.
- 5 - CAMPBELL (AD), TURNER (AW) 1953 - Studies on contagious bovine pleuropneumonia of cattle. IV. An improved complement fixation test. Aust. Vet. J., 29: 154-163.
- 6 - Dannacher (G.), Perrin (M) MARTEL (M.), PERREAU (P.), LE GOFF (C.) - 1986 - Report of Evaluation of the European comparative trial Regarding complement fixation test for diagnosis of contagious bovine pleuropneumonia. Annals Rech. Vet. 17: 107-114.

- 7-Diallo S. Keita., CONDE M.C. Yattara and F. (1999). Sero-surveillance of rinderpest and contagious bovine pleuropneumonia in Guinea. The sero monitoring and surveillance of rinderpest THROUGHOUT Africa. Phase III: Results for 1998, FAO-IAEA-OAU-IBAR-PARC, Machakos, Kenya.
- 8 - DREXLER HG., UPHOFF CC (2002) - *Mycoplasma* contamination of cell culture. Incidence, sources, effects, detection, elimination, prevention Cytotechnology, 39: 75-90.
- 9 - Directorate of Livestock - Dakar 1986: Report of the Committee Epidemio-surveillance of CBPP – DIREL.
- 10 - EMVT - CIRAD: *Mycoplasma* and *Mycoplasma* ruminants. Document technical service infectious disease. June 1985.
- 11 - J HUDSON R., contagious bovine pleuropneumonia. FAO Rome. 1972.
- 12 - J. Mr. Sarr & Diop 2006 Serosurveillance of rinderpest in Senegal: Annual Report.
- 13 - H. Jung, SY Wang., DING WH et al (2003) - Detection and treatment of *Mycoplasma* contamination in cultured cells. Chang. Gung Med J 26: 250-258.
- 14 - LE GOFF C. and THIAUCOURT F., 1998 - A competitive ELISA for the specific diagnosis of contagious bovine pleuropneumonia (CBPP), Vet. Microbiol, 60, 179-191.
- 15 - Minor, L. (1989) - Medical bacteriology, 2nd edition.
- 16 - MBENGUE Mb., J. Sarr, KONTE M. - 2007 Sero - monitoring of contagious bovine pleuropneumonia in Senegal: Annual Report.
- 17 - MBENGUE Mb., THIONGANE Y., FT TALL., LY A B - 2008 Sero - monitoring of contagious bovine pleuropneumonia in Senegal: Annual Report.
- 18 - Manual of standards for diagnostic tests and vaccines. (1992) Office International for Epizooties (12 rue de Prony, 75017 Paris, France), ISBN 92-9044 - 314 - 6, p: 47-56.
- 19 - MATTSSON JG., KE JOHANSSON - 1993 Oligonucleotide probes complementary to

16 S rRNA for rapid detection of *Mycoplasma* contamination in cell cultures FEMS Microbiol Lett. 107: 139-144.

20 - OIE – *Mycoplasmosis* in ruminants. In: Scientific and Technical Review of the OIE., 1987, 6 (3).

21 - PROVOST A., P. Perreau, BREARD A., C. Le Goff, JL MARTEL., Cottew GS - Contagious bovine pleuro pneumonia, REV.OFF.int.epizoot. (1987) 6: 625-679.