Sero-epidemiological studies on contagious bovine pleuropneumonia (CBPP) in Senegal

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ABSTRACT

The sero- prevalence against PPCB is analysed by competition ELISA and CFT tests. Sero – epidemiological results realized on bovine, give the presence of a beginning infection with 0.43% of prevalence rate. The two serological methods have given comparable results. We have noted that some parameters like age, sex and race effects have an important effect on the prevalence rate. We have seen also that aged females zebu cattle have got the most *Mycoplasma mycoides subsp. Mycoides* antibodies prevalence rate.

Keyswords: contagious bovine pleuropneumonia, complement fixation test, cELISA, epidemiology, cattle, Senegal

{**Citation:** Mbengue MB., J. Sarr, Fall Massal. Sero - epidemiological studies on contagious bovine pleuropneumonia (CBPP) in Senegal. American Journal of Research Communication, 2013, 1(3): 190-199} www.usa-journals.com, ISSN : 2325-4076.

INTRODUCTION

Bovine pleuropneumonia (CBPP) is a contagious disease affecting cattle, caused by a *Mycoplasma mycoides subsp. mycoides Small* Colonies SC (*MmmSC)*. This disease is mainly characterized by respiratory symptoms: cough, nasal discharge, dyspnea, tachypnea and the presence of lesions from exsudative pleurisy and pneumonia. The transmission of CBPP is acquired through direct contact or by the excretory products from infected animals and healthy animals. *Mycoplasma* are responsible for conditions on the sphere respiratory, genital, joints and are important pathogenicity for animal in order to decrease

production (10). The presence of CBPP from the suspect animal can be confirmed in the laboratory by a direct method for isolation and identification of the causative agent from the observed lesions, or by indirect serological method including the detection of the possible presence of anti-M mmSC. In order to insure it's eradication a program for sero epidemiological surveillance of the disease was established. In the same area, the research for anti-CBPP was performed on sera collected previously as part of the sero-surveillance for rinderpest now eradicated (Sarr and Col.) [13]. The CBPP is in the way for eradication in Senegal (M. Mbengue, J. Sarr, M. Konté, 2007; M. Mbengue, Y. Thiongane, F. Tall & A.B. Ly, 2008). Thus Serum collected from each one of the region in Senegal (11 regions in total) in the project for CBPP survey have been transferred to the National laboratory for Research on Animal Diseases (LNERV) in order to detect eventually the CBPP anti bodies amount by Competition ELISA which has been recognize for it's great sensitivity by the International Organization for animal diseases (OIE) in order to establish the prevalence of the disease. And to know what we have to do to stop the disease in our countries and in the neighbouring countries.

MATERIALS AND METHODS

1. Animals and experimental protocol

This study was conducted in aged cattle (n= 1114, with 32.7% of males and 67.3% females), from January to August with different races (mainly zebu (71.5%) and Ndama (28.5%)) in extensive farming in various regions of Senegal specially in risk areas:

Figure I: the main risk areas of CBPP in Senegal (see legend). These are mainly the southern zone (Ziguinchor and Kolda), the Eastern Zone (Tambacounda and Kédougou), the Central zone (Kaolack) and the North Zone (Rosso Senegal not far from Mauritania): the map of Senegal with 24 villages during the post winter (October 2006) with the main risk areas are included (seeFig.1). A blood sample was performed for each animal from the jugular vein in a dry vacutainer tube containing 10 ml. After blood coagulation (2 to 3 hours at 25° C), samples were centrifuged in cold at 500g for 15minutes. Sera obtained were stored in plastic tubes of 2 ml per fraction at -20 ° C until serological analysis.

2. Serological reactions

The methods traditionally used (Campbell and Turner, [5] have been replaced by the competitive ELISA and complement fixation reaction (CIRAD Pourquier Kits). As for the

competitive ELISA (kit reference CIRAD CBPP serum competition ELISA Version: P05410 / 01 - / 06/2006.) (Le Goff and Thiaucourt [9], and LEFEVRE Regall [12], sera or positive or negative reference sera provided by the manufacturer (reconstituted with distilled water and stored at - 20 ° C) were diluted 1 / 200 and incubated with the presence of the antigen (purified lysate of the bacteria *Mycoplasma mycoides subsp . mycoides*) adsorbed on the walls of wells and a monoclonal antibody (117 / 5) diluted 1 / 120 for 1 hour at 37 ° C in an oven, followed by washes. After addition of conjugate (bovine sera IgG anti peroxidase-labeled) diluted 1 / 100 and incubation (30 min at 37 ° C) followed by washes which are performed with a washing solution 20X concentrated in the kit to be diluted in 1900 ml of distilled water. Sera washing is conducted after emptying the contents of the plate and filling the wells with the indicated solution: This operation is repeated once (ie 2 washes in total). The washing of the conjugate is the same way with additional (ie total of 3 washes necessary to remove all traces of conjugate that could cause false reactions.). The formation of immune complexes (antigen - monoclonal antibody - conjugated) was revealed by adding a ready solution for use of TMB (Tetra Methyl Benzidine) and we stop the color reaction by adding a solution of H2SO4 0 , 5M. The absorbance of the wells is inversely proportional to the amount of anti-*MmmSC* present in the sample and was read using a microplate reader Multiskan MS Labsystem at the wavelength λ of 450 nm. The samples were considered positive when the percentage inhibition (PI) of the formation of immune complexes was equal or greater than 50%. Anti-*MmmSC* were also confirmed by the complement fixation reaction (CFT kit CIRAD / RFC - CBPP - 1000 - 02230085 ref - lot CF - CBP - 03.) (DANNACHER et al., [6]. The animals sera to be tested has been diluted 1:10, 1:20, and 1:40 or reference sera diluted to the positive or negative 1:10 to 1:1280 were incubated in the presence of antigen (*Mycoplasma mycoides subsp. Mycoides* bacteria lysate) inactivated, purified and containing 2 units at 37 °C for 10 minutes with gentle stirring. After adding a suspension of lyophilized guinea pig complement titrated in 1 / 33, hemolytic sera containing high concentrations of specific antibodies to red blood cells are lysed in vitro when in contact with sera in the presence of hemolytic complement and a suspension of sheep erythrocytes at 3% followed by incubation at 37 °C for 30 minutes with vigorous stirring, the reaction is considered positive when hemolysis inhibition was observed for dilution or greater 1 / 10.

3. Statistical analysis:

The results were analyzed by a χ^2 test to evaluate differences in CBPP prevalence rate based on race, gender and age. Differences were considered significant with a risk of 5%.

RESULTS

In total, six samples gave a positive ELISA reaction and five were considered positive by complement fixation reaction, which corresponds to the sero prevalence of 0.54% and 0.45% respectively. The complement fixation reaction was also positive for 3 of 5 sera diluted 1 / 20. Meanwhile, the sero prevalence of CBPP on the scale of a herd (proportion of herds with at least one animal tested positive) determined by ELISA and complement fixation test were respectively 5.10% and 5 ,10% (Table I). The relative sensitivity of the reaction of complement fixation test compared to ELISA (proportion of negative results by the complement fixation and positive by ELISA) was 66.67%, the relative specificity (proportion of positive results by setting complement and negative by ELISA) of 99.91% and approval between the two methods (proportion of identical scores) of 99.7%. Positive predictive values and negative complement fixation compared with the results of the ELISA are 80% and 99.8% (TableII). The prevalence of CBPP was zero in 6 regions (Fatick, Matam, Saint-Louis, Tambacounda, Thies and Dakar) of the 11 regions explored, while positive results, at least by the ELISA test were obtained in five other regions (Table III).

Figure I: location of the main risks areas of CBPP in Senegal.

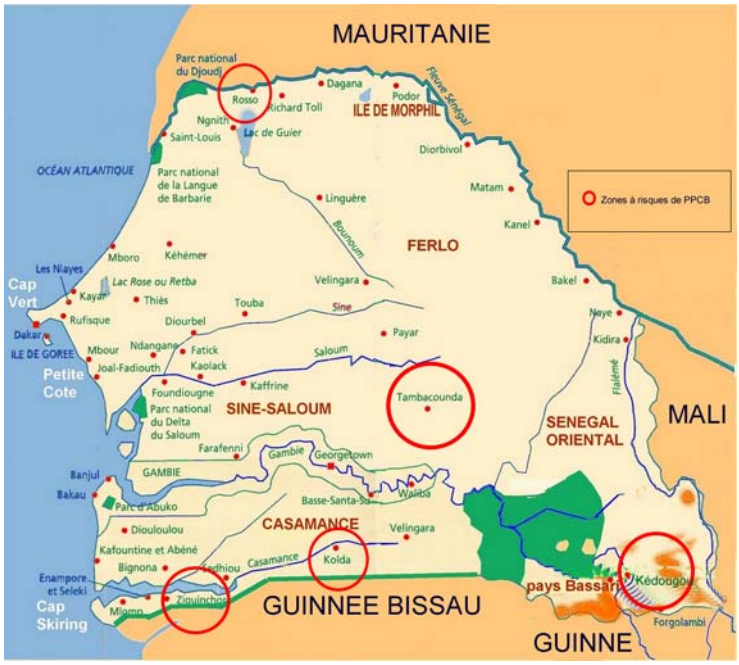

Table I: Seroprevalence of CBPP (contagious bovine pleuro pneumonia) per animal per herd determined by ELISA and complement fixation tests from 1114 cattle herds located in 98from 24 villages in Senegal

	Positive animal s numbers (n = 1114)	Seroprevalence per animal (%)	Herd positive number (n = 98)	Séroprevalence per herd(%)
ELISA	6	0.54	5	5,10
CFT	5	0.45	5	5,10

CFT : Complement Fixation Reaction

Table II: Comparison of two serological tests (competitive ELISA,complement fixation reaction) to detect antibodies MmmSC(*Mycoplasma mycoides subsp. Mycoides*) in the detection of CBPP (contagious bovine pleuro pneumonia) from 1114 cattle

	Negative ELISA	Positive ELISA	FP (%) et Sp (%) FN (%) et Se (%)	VPN (%) VPP (%)
Positive CFT	1	4	FP : 0.09% - Sp : 99.91%	VPN : 99.8%
Negative CFT	1 107	2	FN : 33.3% - Se : 66.67%	VPP : 80.0%
Total	***1 108***	***6***	Agrément : 99.7%	

CFT: complement fixation test, **FP**: false positive; **Sp**: Specificity (probability of obtaining a complement fixation reaction negative in all tested negative in ELISA); **FN**: false negative; **Se**: Sensitivity (probability to get a positive complement fixation tests on all positive ELISA); **VPN**: negative predictive value (probability of obtaining a complement negative fixation reaction); **PPV**: positive predictive value (probability of obtaining a positive complement fixation test).

Table III: Seroprevalence of CBPP (contagious bovine pleuropneumonia) determined per animal per herd by means of ELISA and complement fixation reaction according to the risk for Senegalese explored regions

Region	Total number for bovine (and herds)	Seroprevalences (%) per animal (and per herd) détermined by ELISA	Seroprevalences (%) per animal (and per herd) détermined by CFT
Dakar	83 (3)	0 (0)	0 (0)
Diourbel	98 (8)	1.02 (8,88)	1.02 (12,15)
Fatick	90 (10)	0 (0)	0 (0)
Kaolack	105 (19)	1.90 (10)	0.95 (5)
Kolda	150 (8)	0.67 (8,38)	0 (0)
Louga	140 (13)	0.71 (9,48)	1.43 (11)
Matam	60 (5)	0 (0)	0 (0)
Saint Louis	124 (5)	0 (0)	0 (0)
Tambacounda	130 (5)	0 (0)	0 (0)
Thiès	98 (17)	0 (0)	0 (0)
Ziguinchor	36 (5)	2.78 (53,60)	2.78 (53,60)

CFT: complement fixation Test

Table IV: Influence of gender, age and race on the prevalence of CBPP (contagious bovine pleuro pneumonia) determined by ELISA and complement fixation test

	Effective	Number of positive results ELISA	CFT	P
Sex				
Males	364	1	1	100
Femelles	750	2	4	50
Age				
Classe 1	110	0	0	100
Classe 2	205	1	1	100
Classe 3	256	1	1	100
Classe 4	543	2	3	75
Race				
Zébu	797	2	4	50
Ndama	317	1	1	100

CFT: complement fixation test; NS: not significant.; P = Percentage

DISCUSSION

The individual and herd sero prevalence determined by two different serological methods (ELISA and complement fixation reaction) in this study appeared very low (<1%) 0.54% (per animal) and 5.10% (by herd) and 0.45% (per animal) and 5.10% (by herd), respectively, by ELISA and complement fixation reaction. These results are consistent with the absence of recorded outbreak of CBPP in Senegal for twenty years (8). This low prevalence rate of the disease in Senegal, could move towards eradication unlike other neighboring countries like Mali where the disease is 4 to 10% and a mortality of 3.8% in endemic areas, 25% chronically infected animals after treatment as well as the silent infections remain a dangerous source of infection to long-term factors favoring the spread of the disease in Mali is the extensive nature of livestock transhumance and antibiotics (3). In Guinea, the prevalence rate of individual CBPP equal to 6.3%, remains high (7). For the 11 risk areas explored following the identification of outbreaks of CBPP in some villages characterized by extensive farming method about epidemiological program - overseeing the direction of breeding in Dakar (8), while 5 regions (Fatick, Matam, Saint Louis, Tambacounda, Thies and Dakar) are completely free of CBPP, while a few cases of infection were observed in 4 regions (Diourbel, Kaolack, Louga, and Ziguinchor) Among these four regions, those of Diourbel, Kaolack and Louga, are neighbors with different climates. The regions of Diourbel and Louga, are the Sahel region between the isohyets 300 to 500 mm with a pseudo-type vegetation shrub steppe during normal rainfall. The region of Kaolack, is the Sudano-Sahelian zone limited by isohyets 500 to 800 mm with a vegetation consisting of grasslands and savannas ephemeral perennial grasses. Ziguinchor region is located in the south part of the country. The Ziguinchor region, is the North Guinean zone itself with an index of rainfall between 1200 and 1850 mm. The vegetation in the southern zone, is composed of a succession of semi dense forest - deciduous, densely wooded savannah and dense dry forests (2). This ecological diversity of these four regions as a corollary the existence of different farming systems, and breeds adapted to local conditions. In the northern part of the country (Diourbel - Louga - Kaolack), devoted long-time ranching and transhumant animals bred zebu cattle are Gobra (Bos indicus). (1). On the southern part of Senegal (Ziguinchor), infested by tsetse vector for animal trypanosomiasis, only maintain it and develop trypanotolerant breeds: Ndama cattle (Bos taurus) with an extensive type of farming and sedentary breeding (1).However, the probability of infection of an animal or a herd remained low: respectively 0.67% to 2.78% and 0.45% to 5.10%.The effects of age sex and race are the real prevalence of CBPP. In our study it is mainly old female zebu breed which are the main carriers of antibodies to *Mycoplasma mycoides*

subsp. Mycoides: These observations corroborate thoses made in Mali by Bashiruddin JB, where old female zebu cattle Bos indicus breed are able to keep their antibodies for along time, with the risk of chronic carriers to be dangerous for the transmission of CBPP in contrast to young calves (3). The two serological methods (ELISA and complement fixation) yielded similar results: although the relative sensitivity of complement fixation test compared for the average or ELISA (66.67%), show the relative specificity and the registration between the two methods were higher than 99.5%. Similarly, the negative predictive value on the complement fixation test compared with the ELISA was very high (99.8%). It appears from these results that obtaining a negative result by complement fixation test increases the probability of excluding a *Mycoplasma* infection *Mycoplasma mycoides subsp.mycoides SC* across a population. In addition, the nature of the two antibodies revealed by serological tests may be different: RFC is used to detect IgM and some IgG subclasses: This technique is valid in terms of observing the criteria of strict construction (presumptive minimum rate of at least 1 / 64). The competitive ELISA test is for antibodies against a protein antigen and used to separate IgM and IgG (4, 15) where the future of this new technique approved by the OIE as well as the RFC as alternative test that can be used to obtain the official results for the CBPP. The complement-fixing antibodies are immunoglobulins of classes M and G and those detected by ELISA as immunoglobulin G: the heterogeneous nature of antibodies and detected by both methods can explain the presence of "false positives" (presence of IgM revealed by complement fixation but not by ELISA: 1 case in the Louga region) and “false negatives” (persistence of IgG revealed by ELISA and little or no ability to fix complement: 2 cases: one in the region of Kaolack, and one in Kolda). Thus the complement-fixing antibody vaccine disappears after a maximum period of three months, while those detected by ELISA may persist in the general circulation over a year (12). Therefore, although vaccination campaigns against CBPP have been carried out in 2005 in the regions of Diourbel and Louga, the presence of anti-complement-fixing CBPP highlighted on three animals can only result from a recent contamination by *Mycoplasma.* In the absence of vaccination in the other regions studied, other serology positive sign has obtained the existence of an incipient infection of CBPP.

CONCLUSION

Although outbreaks of CBPP seem to have disappeared in Senegal for several years, carrying the few cases of anti-CBPP revealed by this study using two different serological tests (complement fixation and ELISA tests) are compatible with situations of recent contamination

by *Mycoplasma mycoides sub sp mycoides* SC and thus reflect a possible resurgence of the disease. It is therefore necessary to investigate more larger on sero epidemiological in order to locate any new outbreaks of CBPP and analyze the inherents risks factors.

REFERENCES

1. ADAM J. G. - 1966: natural pastures and crop post from Senegal Bulletin IFAN, T. XXVIII, Series A. pp. 450-537

2. AUBREVILLE A. - 1977: Climate, desertification and forests of tropical Africa. Paris, Editions Companies maritime and colonial geography, 1949. 351p.

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, biotechnology and Agriculture, Bern, p.150-154.

4. BUSOLO T., CONVENTIN. C. - 1980: Enzyme - linked immunosorbent assay for detection of *Mycoplasma pneumoniae* antibodies. J. Clin. Microbiol. 12, 69-73.

5. CAMPBELL AD, TURNER AW: Studies on contagious bovine pneumonia of cattle wept. IV. An improved complement fixation test. Aust. Vet. J., 1953, 29: 154-163.

6. DANNACHER G., PERRIN M., M. MARTEL, PERREAU P., Le Goff C.: - Report of Evaluation of the European comparative trial Regarding complement fixation test for diagnosis of contagious bovine pneumonia wept. Ann. Rech. Vet., 1986, 17: 107-114.

7. DIALLO S. KEITA., CONDE M.C. and Yattara F. (1999). Sero-surveillance of rinderpest and contagious bovine pleuropneumonia in Guinea. The monitoring and sero surveillance of rinderpest throughout Africa. Phase III: Results for 1998, FAO-IAEA-OAU-IBAR-PARC, Machakos, Kenya.

8. Directorate of Livestock - Dakar 1986: Report of the epidemiological surveillance of CBPP – DIREL.

9. LE GOFF C., F. Thiaucourt: A competitive ELISA for the diagnosis of contagious bovine Specific wept pneumonia (CBPP), Vet. Microbiol., 1998, 60: 179-191.

10. Le Minor, L. (1989) - Medical Bacteriology, 2nd edition.

11. Palya V. Rweyemamu & MM (1992: Manual of standards for diagnostic tests and vaccines. Office International des Epizooties (12 rue de Prony, 75017 Paris, France), ISBN 92-9044 - 314 - 6, 1992, pp: 47-56.

12. Regall J., LEFEVRE PC - 2000: Contagious bovine pleuropneumonia (Chapters 2, 4, 6),

in Manual of standards diagnostic tests and vaccines of OIE, 12 rue de Prony 75017 Paris France, pp: 503-514.

13. J. CARS, DIOP M. - 2004: Monitoring of rinderpest in Senegal in the annual report, PACE 2005, ISRA - LNERV - LMPA, 80p.

14. J. Sarr, M Diop 2007.: Sero-surveillance of rinderpest in Senegal: Annual Report, 2006 PACE, ISRA - LNERV - LMPA, 90p.

15. G. VAN, AJA D., R. Graaf, J. Druten, 2000: Use of Enzyme - linked immunosorbent assay for early diagnosis of Mycoplasma pneumoniae infections. Eur. J. Clin. Microbiol 3: 116-121.